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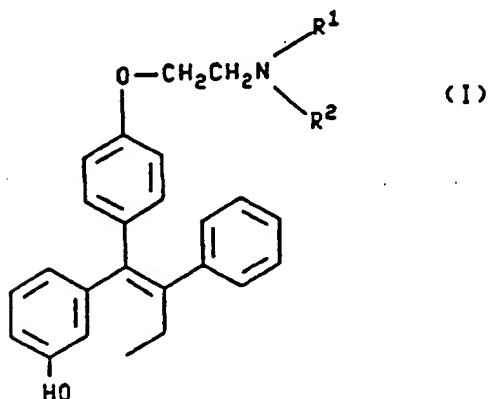
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(54) Use of (E)-1-(4'-(2-alkylaminoethoxy)-phenyl)-1-(3'-hydroxyphenyl)-2-phenylbut-1-enes for inhibiting pathological conditions

(57) The use of a compound of the formula (I) for the manufacture of a medicament for inhibiting a condition selected from pathological conditions related to organ systems which respond to estrogen agonists, uterine fibrosis, myeloperoxidase activity, autoimmune diseases, reperfusion damage in ischemic myocardium, and the symptoms of premenstrual syndrome:-

wherein

R<sup>1</sup> and R<sup>2</sup> may be the same or different provided that, when R<sup>1</sup> and R<sup>2</sup> are the same, each is a methyl or ethyl group, and, when R<sup>1</sup> and R<sup>2</sup> are different, one of them is a methyl or ethyl group and the other is hydrogen or a benzyl group.

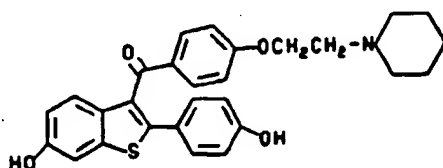


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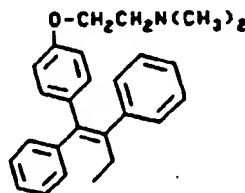
## Description

## BACKGROUND OF THE INVENTION

Certain estrogen agonists have been reported to be useful in inhibiting pathological conditions related to organ systems which respond to estrogen agonists or antagonists. In particular, 2-phenyl-3-arylbenzothiophenes and 1-(alkylaminoethoxy phenyl)-1-phenyl-2-phenylbut-1-enes represented by raloxifene and tamoxifen have wide application as estrogen agonists.



Raloxifene



Tamoxifen

Raloxifene has been claimed to be effective in the treatment of acne, U.S. 5,439,923; alopecia, EP 0659414 A2; Alzheimer's disease, EP 0659418 A1; atrophy of skin and vagina, US 5,461,064; autoimmune disease, EP 0664123; breast cancer, US 4,418,068; breast disease, EP 0659419; cartilage degeneration, US 5,418,252; CNS problems (post menopausal), 94 EP 0309470; pathology of endocrine target organs, US 4,418,068; delayed puberty, US 5,451,589; demyelinating disease, US 5,434,166; dysmenorrhea, US 5,446,053; endometriosis, US 5,461,065; female infertility, EP 659429 A1; fertility disorders; hirsutism, EP 0659414 A2; hypoglycemic, EP 635264 A2; increase libido, US 5,439,931; inhibition of fertility, US 5,462,949; LDL oxidation, EP 0664121 A; hypercholesterolemia, US 5,464,845; lupus erythematosus, EP 0664125; impaired macrophage function, EP 659425 A1; male infertility, EP 0659424 A1; myocardial infarction, ischaemia, thromboembolic disorder, thrombin inhibition, EP 0664126; menopausal disorders, EP 0659415; menstruation disorders, US 5,462,950; obesity, 94 EP 0309481; obsessive compulsive disorder, EP 0659428; osteoporosis, US 5,457,117; ovarian dysgenesis, US 5,451,589; peri-menopausal syndrome, US 5,391,557; peripheral vasoconstriction, EP 0659415; premenstrual syndrome, US 5,389,670; prostatic carcinoma; prostatic hyperplasia; pulmonary hypertension, US 5,447,941; reperfusion damage, J. AM. Cardiol 25, 189A (1993); resistant neoplasm, EP 0652004 A1; restenosis, US 5,462,937; rheumatoid arthritis, EP 0664125; seborrhea, US 5,439,923; sexual dysfunction; sexual precocity, US 5,451,590; thrombomodulin expression, EP 0659427; Tumors syndrome, US 5,441,966; uterine fibrosis US 5,457,116; and vasomotor symptoms (post menopausal), 94 EP 0309473.

Tamoxifen is widely employed in the treatment of breast cancer and has been reported to be effective in the treatment of the following diseases and conditions: high lipid levels, Drug Ther. 22/3, 109 (1992); ovarian cancer, J. Clin. Oncol. 11, No. 10, 1957-68 (1993); renal cell carcinoma, Br. J. Radiol 56, No. 670, 766-7 (1983); suppression of atherogenic factor homocysteine, Env. J. Cancer 29 Suppl. 6, S110 (1993); metastatic melanoma, J. Clin. Oncol. 12, No. 8, 1553-60 (1994); mastalgia, Drugs 32, No. 6, 477-80, (1986); prolactin secreting pituitary tumors, J. Endocrinol. Invest. 3/4, 343-347 (1980); osteoporosis, Proc. Annu Meet Am Assoc. Cancer Res., 33: A566-7 (1992); retroperitoneal fibrosis, Lancet 341, No. 8841, 382 (1993).

Small structural changes in the structure of estrogen agonists cause profound differences in biological properties. For example, droloxifene (3-hydroxytamoxifen) formula I below, has a 10-60-fold higher binding affinity to the estrogen receptor compared to tamoxifen. Droloxifene is devoid of *in vivo* or *in vitro* carcinogenic or mutagenic effects, whereas tamoxifen causes liver tumors in rats. Haslam, et al. Cancer Letters 84, 101-116 (1994).

Droloxifene has been reported to be effective in the treatment of breast cancer US 5,047,431; endometriosis, US 5,455,275; lowering cholesterol, US 5,426,123; osteoporosis, US 5,254,594; prostatic hyperplasia, US 5,441,986; and restenosis, US 5,384,332.

Each month, for a few days prior to the onset of menstruation, many millions of otherwise-healthy women develop symptoms of disturbed mood and appetite that can be strikingly similar to those reported by patients with Seasonal Affective Disorder (SAD), carbohydrate-craving obesity, or the non-anorexic variants of bulimia. This syndrome was first termed "premenstrual tension" by R. T. Frank in 1931 and is a very common phenomenon. According to Guy Abraham of UCLA, of every ten patients to walk into a gynecologist's office, three or four will suffer from premenstrual tension and, in some, the symptoms will be of such severity as to include attempts at suicide. Current Progress in Obstetrics and Gynecology, 3:5-39 (1980).

Initial descriptions of the Premenstrual Syndrome (PMS) focused on its association with nervous tension, headache, and weight gain. The weight gain observed was initially attributed to excessive retention of salt and water, which does indeed occur in some PMS patients. However, it soon became evident that it was also a consequence of the widespread tendency of individuals suffering from PMS to crave and overconsume carbohydrates, particularly foods with a sweet taste. PMS is also now referred to as late luteal phase syndrome (or late luteal phase dysphoric disorder).  
 5 D.N.S. III, Revised, American Psychiatric Association (1987).

There have been numerous suggestions made about the etiology of PMS. For example, some hypothesized that it was caused by a uterine toxin. Others suggested its cause was overconsumption of sweets, which was presumably followed by excessive insulin secretion, hypoglycemia, and inadequate brain glucose, and resulted in the often observed depression and anxiety. It also has been postulated that the behavioral symptoms result from the tissue edema often observed and that the psychological changes result from feelings of loss or the social complexities generated by the discomforts of menstruation.

However, none of these theories has been substantiated: PMS can persist after hysterectomy and, hence, uterine toxins cannot be its cause; the hyperinsulinism of PMS is not associated with low blood glucose levels, and is probably the consequence of a behavioral aberration (i.e., the tendency of premenstrual women to choose high-carbohydrate diets, which potentiate insulin secretion) rather than the cause; the mood and appetitive changes of PMS are poorly correlated with the tissue swelling; and subhuman primates who are presumably exempt from the psychodynamic or social complexities of human life also exhibit characteristic behavioral changes premenstrually.

There have been many treatments suggested for overcoming or reducing the symptoms of PMS. These include carbohydrate-free diets, vitamin supplements, ovarian hormones, detoxifying agents, irradiation of the ovaries and pituitary, and use of diuretics. These approaches have all had limited success, however.

Late Luteal Phase Dysphoric Disorder (LLPDD) is the current term associated with Premenstrual Syndrome (PMS). Many females report a variety of physical and emotional changes associated with specific phases of the menstrual cycle. For most of these females, these changes are not severe, cause little distress, and have no effect on social or occupational functioning. In contrast, the essential feature of LLPDD is a pattern of clinically significant emotional and behavioral symptoms that occur during the last week of the luteal phase and remit within a few days after the onset of the follicular phase. In most females, these symptoms occur in the week before and remit within a few days after the onset of menses.

LLPDD is diagnosed only if the symptoms are sufficiently severe to cause marked impairment in social or occupational functioning and have occurred during a majority of menstrual cycles in the past year.

Among the most commonly experienced symptoms are marked affective lability (e.g., sudden episodes of tearfulness, sadness, or irritability), persistent feelings of irritability, anger, or tension, feelings of depression, and self-deprecating thoughts. Also common are decreased interest in usual activities, fatigability and loss of energy, a subjective sense of difficulty in concentration, changes in appetite, craving for specific foods (especially carbohydrates), and sleep disturbance. Other physical symptoms, such as breast tenderness or swelling, headaches, joint or muscle pain, a sensation of bloating, and weight gain, also may be present.

Generally, non-steroidal anti-inflammatory drugs are administered to LLPDD patients, but these only are effective for some of the physical symptoms. The physical manifestations of PMS, if severe, may be treated symptomatically. Water retention may be relieved by diet or antidiuretic medication, but severity of water retention does not always correlate with psychological symptoms. Recent studies have suggested that spironolactone (Aldactone, Searle) may also be effective in relieving depression and crying spells.

Other drugs, including progesterone, lithium carbonate, thiazide, diuretics, antidepressants and bromocriptine (Parlodel®, Sandoz), have been tried with uncertain success.

United States Patent 5,389,670 describes the use of certain benzothiophenes for treatment of LLPDD/PMS.

In view of the drawbacks and inadequacies with existing methods of treating PMS/LLPDD, new therapies are sought.

Uterine fibrosis is an old and ever present clinical problem which goes under a variety of names, including uterine hypertrophy, uterine leiomyomata, myometrial hypertrophy, fibrosis uteri, and fibrotic metritis. Essentially, uterine fibrosis is a condition where there is an inappropriate deposition of fibroid tissue on the wall of the uterus.

This condition is a cause of dysmenorrhea and infertility in women. The exact cause of this condition is poorly understood but evidence suggests that it is an inappropriate response of fibroid tissue to estrogen. Such a condition has been produced in rabbits by daily administrations of estrogen for 3 months. In guinea pigs, the condition has been produced by daily administration of estrogen for four months. Further, in rats, estrogen causes similar hypertrophy.

The most common treatment of uterine fibrosis involves surgical procedures both costly and sometimes a source of complications such as the formation of abdominal adhesions and infections. In some patients, initial surgery is only a temporary treatment and the fibroids regrow. In those cases a hysterectomy is performed which effectively ends the fibroids but also the reproductive life of the patient. Also, gonadotropin releasing hormone antagonists may be administered, yet their use is tempered by the fact they can lead to osteoporosis.

United States Patent 5,457,116 describes a method for inhibiting uterine fibrosis by treatment with certain benzothio-  
 thiophene compounds.

Autoimmune diseases involve aberrant regulation of cellular and humoral mediated immunity and are frequently associated with abnormal or enhanced T cell, B cell and macrophage effector functions directed towards self antigens. The activation of these cellular components towards self antigens is believed related to the break in feedback mechanisms associated with self tolerance. Autoimmune diseases encompass a whole spectrum of clinical entities and despite the differences in the target organ have many similarities. These include their preponderance in females of child bearing age with a female to male ratio varying from 50:1 in Hashimoto's thyroiditis to 10:1 in Systemic lupus erythematosus (SLE) to 2:1 in Myasthenia gravis (Ahmed et al., Am J. Path., 121:531 (1985)). In addition, these diseases are all characterized by the chronicity, the tendency of clinical remission and "flare ups" for poorly understood reasons, and the involvement of other organs. While the presence of autoantibodies, inappropriate expression of class II antigens, macrophage activation and T cell infiltration to the target organ have been described in essentially all of the autoimmune diseases, neither the triggering mechanisms which result in disease activation nor disease progression are well understood. Accordingly, therapy for these diseases is largely unsatisfactory and involves the use of gold salts, methotrexate, antimalarials, glucocorticoids (methylprednisolone), and other immunosuppressives as well as plasmapheresis and attempts at inducing tolerance. Treatment of autoimmune diseases has not improved significantly over the past decade and primarily is associated with the use of nonsteroidal and steroidal anti-inflammatory agents to treat the symptoms of the disease. Clearly while suppression of the specific immune response directed against the host is necessary, generalized immunosuppression as with glucocorticoids has major liabilities in terms of side effect profile and the propensity of the immunosuppressed patient to be at greater risk for other infectious and non-infectious diseases.

Polymorphonuclear leukocytes (PMNL) play a regulatory role in inflammatory diseases. These cells, when activated, synthesize and release oxygen-centered molecules, chemo-attractants, and hydrolytic enzymes. There is evidence that the oxygen-centered molecules play a detrimental role in a number of diseases such as chronic inflammatory diseases, rheumatoid arthritis, SLE, and others. In the case of an autoimmune disease, SLE, for example, the initiation of an inflammatory response are self antigen stimulating one's host neutrophils or PMNLs to secrete strong oxidants which damage surrounding cells and tissue.

Estrogen appears to be involved with autoimmune diseases although its role in disease progression or regression is complex and dependent on the nature of the autoimmune disease. Estrogen for example appears to have ameliorating effect on rheumatoid arthritis while having an exacerbating effect on systemic lupus (Chander & Spector; Ann. Rheum. Dis. 50:139). As reported by Jansson (Free Rad Res Comms, 14(3), 195-208, (1991), incorporated herein by reference), estrogen increased the activity of an enzyme generated by PMNLs, myeloperoxidase, which regulates the production of oxidants from hydrogen peroxide. This enzyme converts hydrogen peroxide to hypochlorous acid, a strong oxidant. By increasing the enzyme's activity, and thus the presence of hypochlorous acid, the likelihood of increased oxidative stress on tissues, cells and various macromolecules in chronic inflammatory/autoimmune diseases is enhanced.

EP 664 125 A1 reports that inhibition of myeloperoxidase may be accomplished by treatment with certain 3-aryl benzothio-  
 thiophenes. Excess myeloperoxidase is associated with conditions which include systemic lupus erythematosus, Hashimoto's thyroiditis, myasthenia gravis, rheumatoid arthritis and multiple sclerosis.

Autoimmune diseases involve aberrant regulation of cellular and humoral mediated immunity and are frequently associated with abnormal or enhanced T cell, B cell and macrophage effector functions directed towards self antigens. The activation of these cellular components towards self antigens is believed related to the break in feedback mechanisms associated with self tolerance. Autoimmune diseases encompass a whole spectrum of clinical entities and despite the differences in the target organ have many similarities. These include their preponderance in females of child bearing age with a female to male ratio varying from 50:1 in Hashimoto's thyroiditis to 10:1 in Systemic lupus erythematosus (SLE) to 2:1 in Myasthenia gravis (Ahmed et al., Am J. Path., 121:531 (1985)). In addition, these diseases are all characterized by their chronicity, the tendency of clinical remission and "flare ups" for poorly understood reasons, and the involvement of other organs. While the presence of autoantibodies, inappropriate expression of class II antigens, macrophage activation and T cell infiltration to the target organ have been described in essentially all of the autoimmune diseases, neither the triggering mechanisms which result in disease activation nor disease progression are well understood. Accordingly, therapy for these diseases is largely unsatisfactory and involves the use of gold salts, methotrexate, antimalarials, glucocorticoids (methylprednisolone), and other immunosuppressives as well as plasmapheresis and attempts at inducing tolerance. Treatment of autoimmune diseases has not improved significantly over the past decade and primarily is associated with the use of nonsteroidal and steroidal anti-inflammatory agents to treat the symptoms of the disease. Clearly while suppression of the specific immune response directed against the host is necessary, generalized immunosuppression as with glucocorticoids has major liabilities in terms of side effect profile and the propensity of the immunosuppressed patient to be at greater risk for other infectious and non-infectious diseases.

Estrogen appears to be involved with autoimmune diseases although its role in disease progression or regression is complex and dependent on the nature of the autoimmune disease. Estrogen for example appears to have an ameliorating effect on rheumatoid arthritis while having an exacerbating effect on systemic lupus (Chander & Spector; Ann. Rheum. Dis. 50:139). Estrogen has been demonstrated to have a suppressive role on T cell function and yet an immunostimulatory effect on B cells. Therefore, estrogen-like compounds should prove beneficial in diseases associated with activated T cells including rheumatoid arthritis, multiple sclerosis, Guillan Barre syndrome and Hashimoto's thyroiditis through inhibition of T cell function (Holmadahl, J. Autoimmun. 2:651 (1989)).

In addition to the suppressive effects of estrogen on T cells, estrogen may have additional protective roles. Marui et al., (J. Clin. Invest. 92:1866 (1993)) have recently reported that antioxidants suppress endothelial expression of VCAM-1. VCAM-1 is the ligand for VLA-4, the T cell and macrophage integrin associated with trafficking of these cells out of the vasculature and into the perivascular space and target organs. As estrogen is an antioxidant, it would be anticipated that estrogen and related analogs will inhibit VLA-4 dependent trafficking of cells and thus hinder the immune cascade associated with autoimmune mediated disease.

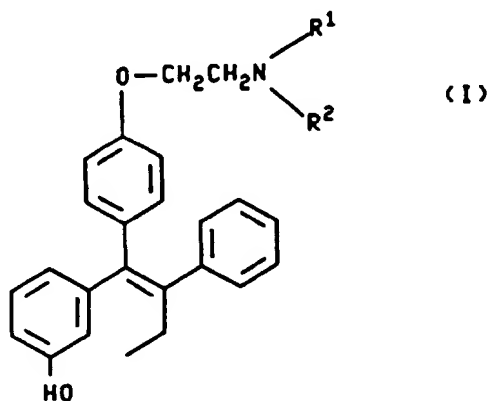
Estrogen plays a detrimental role in other autoimmune diseases including systemic lupus and glomerulonephritis, diseases associated with immune complexes. While the mechanism(s) responsible for estrogen mediated disease progression are not known, the ability of estrogen to increase Fc mediated phagocytosis (Friedman et al., J. Clin. Invest. 75:162 (1985)), and class II antigen expression and IL-1 production by macrophages from estrogen treated rodents (Flynn, Life Sci., 38:2455 (1986)) has been reported. Enhancement of these macrophage mediated effector functions would be expected to contribute towards the immune cascade associated with self destruction.

It is reported in EP 664123 A1 that certain 2-phenyl-3-arylbenzothiophenes are effective inhibitors of autoimmune diseases.

Premenopausal women are at lower risk of coronary heart disease than their male counterparts, and it has been shown that estrogen treatment protects against cardiovascular disease in postmenopausal women. Hale and Kloner have shown that acute pretreatment with estradiol reduced myocardial infarct size caused by occlusion of the coronary artery in both male and female rabbits, thus demonstrating that estrogen exerts a protective effect on ischemic myocardium that is not associated with an increase in myocardial blood flow or alteration in neurodynamics. J. Am. Coll. Cardiol. 25, 189A (1995).

### SUMMARY OF THE INVENTION

The present invention provides a method of inhibiting a pathological condition which is susceptible or partially susceptible to inhibition by an estrogen, antiestrogen or estrogen agonist, which comprises administering to a mammal suffering from a pathological condition selected from the group consisting of uterine cancer, adjuvant breast cancer, breast disorder, male breast cancer, migraine, incontinence, vaginal atrophy, bladder infection, senile gynecomastia, diabetes, hypoglycemia, failure of wound healing, melanoma, impotence, inflammatory bowel disease, CNS and GI disorders caused by an excess of tachykinins, decreased libido, immune system disorders, decreased fertility, pulmonary hypertensive disease, acne, seborrhea, autoimmune disease, Turner's syndrome, alopecia, hirsutism, disorders related to an excess of neurokinin and obsessive-compulsive disorders including smoking and alcohol abuse, an effective dose of a compound of formula I



wherein

R<sup>1</sup> and R<sup>2</sup> may be the same or different provided that, when R<sup>1</sup> and R<sup>2</sup> are the same, each is a methyl or ethyl group, and, when R<sup>1</sup> and R<sup>2</sup> are different, one of them is a methyl or ethyl group and the other is hydrogen or a benzyl

group; or a pharmaceutically acceptable salt thereof. A preferred compound of formula I is the compound in which R<sup>1</sup> and R<sup>2</sup> are methyl. A preferred salt is the citrate salt.

In another aspect this invention provides a method for inhibiting the symptoms of premenstrual syndrome comprising administering to a human in need of treatment an effective amount of a compound of formula I.

5 wherein

R<sup>1</sup> and R<sup>2</sup> may be the same or different provided that, when R<sup>1</sup> and R<sup>2</sup> are the same, each is a methyl or ethyl group, and, when R<sup>1</sup> and R<sup>2</sup> are different, one of them is a methyl or ethyl group and the other is hydrogen or a benzyl group;

or a pharmaceutically acceptable salt thereof.

10 This invention also provides a method for inhibiting uterine fibrosis comprising administering to a mammal in need of such treatment an effective amount of a compound of formula I wherein

R<sup>1</sup> and R<sup>2</sup> may be the same or different provided that, when R<sup>1</sup> and R<sup>2</sup> are the same, each is a methyl or ethyl group, and, when R<sup>1</sup> and R<sup>2</sup> are different, one of them is a methyl or ethyl group and the other is hydrogen or a benzyl group;

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or a pharmaceutically acceptable salt thereof.

This invention also provides a method for inhibiting myeloperoxidase activity comprising administering to a mammal in need of such treatment an effective amount of a compound of formula I wherein

20 R<sup>1</sup> and R<sup>2</sup> may be the same or different provided that, when R<sup>1</sup> and R<sup>2</sup> are the same, each is a methyl or ethyl group, and, when R<sup>1</sup> and R<sup>2</sup> are different, one of them is a methyl or ethyl group and the other is hydrogen or a benzyl group;

or a pharmaceutically acceptable salt thereof.

25 This invention also provides a method for inhibition of autoimmune diseases comprising administering to a mammal in need of treatment an effective amount of a compound of formula I wherein

R<sup>1</sup> and R<sup>2</sup> may be the same or different provided that, when R<sup>1</sup> and R<sup>2</sup> are the same, each is a methyl or ethyl group, and, when R<sup>1</sup> and R<sup>2</sup> are different, one of them is a methyl or ethyl group and the other is hydrogen or a benzyl group;

30 or a pharmaceutically acceptable salt thereof.

This invention also provides a method for inhibiting reperfusion damage in ischemic myocardium comprising administering to a mammal in need of such treatment an effective amount of a compound of formula I wherein

35 R<sup>1</sup> and R<sup>2</sup> may be the same or different provided that, when R<sup>1</sup> and R<sup>2</sup> are the same, each is a methyl or ethyl group, and, when R<sup>1</sup> and R<sup>2</sup> are different, one of them is a methyl or ethyl group and the other is hydrogen or a benzyl group;

or a pharmaceutically acceptable salt thereof.

#### DETAILED DESCRIPTION OF THE INVENTION

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The present invention concerns methods for inhibiting pathological conditions which are susceptible or partially susceptible to inhibition by an estrogen, antiestrogen or estrogen agonist. Such conditions include uterine cancer, adjuvant breast cancer, breast disorder, male breast cancer, migraine, incontinence, vaginal atrophy, bladder infection, senile gynecomastia, diabetes, hypoglycemia, failure of wound healing, melanoma, impotence, inflammatory bowel disease, CNS and GI disorders caused by an excess of tachykinins, decreased libido, immune system disorders, decreased fertility, pulmonary hypertensive disease, acne, seborrhea, autoimmune disease, Turner's syndrome, hirsutism, disorders related to an excess of neurokinin and obsessive-compulsive disorders including smoking and alcohol abuse.

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Changes in the appearance and texture of the skin with increasing age has been proverbial and well documented both quantitatively and qualitatively. It is a subject which is highly subjective in its evaluation and its ultimate effect on the individual. By and large the effect of the general atrophy of skin with age is cosmetic, but can have pathological consequences, many of which are psychological in nature, i.e., the feeling of getting "old," depression, loss of sexual attractiveness, etc. In some cases, the atrophy of the skin in older people can have direct pathologies associated with it, e.g., the ability of the skin to repair in wound healing. In general, the atrophy of the skin is considered a normal and progressive consequence of the aging process and taken with "good grace." Despite the normal acceptance of aging, there is a particular time in a woman's life, i.e., the menopause, when the progressive aging pattern is greatly accelerated, especially with regard to atrophy of the vagina and skin. It is often this rapid acceleration and suddenness of change which can be contributory to pathological and psychological distress. Additionally, vaginal atrophy can lead to

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discomfort, e.g., itching, dryness, and painful intercourse, which can lead to a loss of sexual enjoyment and conjugal harmony and in some cases be causal in social sequelae such as divorce.

As mentioned before, the atrophy or aging of the skin can have both qualitative and quantitative aspects. The qualitative aspects are: the change of smoothness and texture, thus causing a "roughness" in look and feel on the outer surface of the skin, the change of elasticity of the skin, thus effecting the mechanical properties of the skin, and the changes in skin pigmentation. These qualitative changes result in the commonly described condition of atrophied skin as: wrinkled, rough, withered, and spotty. Quantitatively, skin aging in post-menopausal women can be measured as: a decrease in the mitotic rate of keratinocytes, changes in dermal thickness, decrease in glycosaminoglycans and soluble collagen which are linked to the moisture content of the skin, and the decrease in the urinary excretion of hydroxyproline, a measure of decrease collagen turnover. The qualitative changes in the skin, i.e., sightlessness and mechanical properties, are the result of the quantitative changes, i.e., loss or change of the extra-cellular matrix components. Therefore, it is possible to evaluate a beneficial effect of a therapy for post-menopausal skin atrophy without totally relying on subjective analysis, even though a subjective improvement may be the ultimate desired effect. In the case of vaginal atrophy, the quantitative aspect is the amount of vaginal moisture which is controlled by the amount of secretion from glands in the dermis, the qualitative result is subjective comfort.

Currently, there are two major therapies available for the treatment of skin and vaginal atrophy in post-menopausal women. The first therapy is strictly a cosmetic approach, e.g., the use of make-up, skin moisturizers, night cremes, vaginal lubricants, etc. Although this cosmetic therapy does not affect the underlying physiological cause of the atrophy, often it does achieve some subjective benefit for the individual. The second type of therapy involves the treatment of the underlying physiological causes with active, medicinal agents, most notably Vitamin A and estrogens. Vitamin A is used, its effectiveness is controversial, and it is known to have substantial, undesirable side-effects which limit its use.

At the time of menopause, the levels of estrogen produced by the ovaries rapidly decrease. This decrease in estrogen has pronounced effects on the skin and vagina causing a rapid acceleration in the natural process of atrophy. Estrogen replacement therapy is often beneficial in treating skin and vaginal atrophy. However, estrogen replacement therapy has undesired side-effects, most serious of which is the potential for the development of cancer. The inclusion of progestinal agents leads to undesirable psychological effects. The use of estrogen replacement therapy for the sole purpose of treating skin and vaginal atrophy is not common because of the negative side-effects. Clearly, an effective and safe agent which positively effects the underlying physiology and thus improves the qualitative aspects of skin and vaginal properties in post-menopausal women would be useful.

Due to the advancements in medical science, along with education, the quality and length of life have both been increased. As a result, the population, as a whole, is living longer. As such, situations which have been present but not in great numbers are becoming more numerous and recognized.

One of those situations which has been present but possibly not given enough weight is sexuality in old age. While there has always been some thought that old people are incapable of engaging in sexual activities, which is somewhat supported by a definite decline in sexuality for both sexes as they get older, sexual activity among the elderly cannot be ignored. Therefore, problems associated with such activity also cannot be ignored.

While the male, from the early and middle years, has a relatively steady decline in sexual capacity and activity, the same cannot be as convincingly said about women. There are reports that the sexual capacity of the female does not change much until late in life. (Kinsey, A.C., et al., *Sexual Behavior in the Human Female*, Saunders, PA., pg. 353 (1953)). In particular, libido decreases substantially in a considerable percentage of cases after the menopause (Lauritzen et al., *Estrogen Therapy the Benefits and Risks*, 3rd International Workshop on Estrogen Therapy in Geneva, Oct. 20-21, 1977, *Frontiers of Hormone Research*, Vol. 5, pg. 10 (1978)). This belief is supported by Pfeiffer, E., et al., *Terminus of Sexual Behavior in Middle and Old Age*, *Journal of the American Geriatric Society*, pg. 2151-2158 (1972), and Pfeiffer et al., *Sexual Behavior in Middle Life*, *American Journal of Psychiatry*, 128, 1262-1267 (1972). In the Pfeiffer studies, a much greater decline in both sexual activity and interest among women between the ages of 45 and 71 was found as compared to men the same age, and the most dramatic change took place between 50-60 years of age, which is, of course, generally the period during which women experience menopause or are post-menopausal. In the age group of 66-71, 50% of women said they had no or little sexual interest compared with 10% of men in the same age group.

While there has been no direct link between declining estrogen levels and declining sexuality, it has been stated that hormonal changes following a natural menopause, and certainly following surgical menopause, may contribute to the sexual decline in a portion of women. Exactly how menopause contributes to the loss of libido is not understood, yet seems fairly evident. (Bancroft, *Human Sexuality and Its Problems*, 2nd ed., pg. 292-293, (1989)). Therefore, it would be of use to find compounds which increase libido in post-menopausal women.

Contraceptive methods involving the administration of chemical substances are widely practiced among women who desire to limit pregnancies. Such methods control fertility through various biological mechanisms. Among the presently used chemical methods of fertility control, the most important are those which act by means of the following: (a) suppression of ovulation through inhibition of gonadotropin release; (b) alteration of the female reproductive tract



to prevent migration of sperm to the site of fertilization or, if fertilization occurs, to block implantation of the zygote (nidation); (c) spermicidal action or (d) an abortifacient.

The oral contraceptives are the most prominent chemical contraceptive agents. Two types of agents are (a) estrogen combined with a progestin, and (b) a progestin alone. The contraceptives of the combined type act primarily by suppressing ovulation by negative feedback to prevent gonadotropin (LH and FSH) release by the hypothalamus, but alterations in the reproductive tract may also contribute to the antifertility effect. Such alterations include changes in the cervical mucus (which increase the difficulty of sperm migration) and in the endometrium (which decrease the likelihood of nidation). The action of a progestin alone in a very low oral dose ("mini-pill") appears to involve primarily alterations in the female reproductive tract, but ovulation suppression may also occur. Although the oral contraceptives are highly effective, their use is associated with unpleasant side effects (such as nausea, depression, weight gain, and headache) and an increased long-time risk of severe disease (such as thromboembolism, stroke, myocardial infarction, hepatic adenoma, gall bladder disease, and hypertension). Bleeding irregularities (such as break-through bleeding, spotting, and amenorrhea) are also frequent. A progestin, when administered alone, causes an increased incidence of changes in menstrual patterns, especially a marked increase in the amount and duration of menstrual bleeding.

Other chemical methods of contraception include the post coital administration of estrogens (e.g. diethylstilbestrol, antiprogesterins, or ethynylestradiol) to prevent nidation or of prostaglandins which act as abortifacients. Both of these methods, at present, are limited to emergency situations. Still in the very early stages of development are immunological methods (vaccination) and methods involving the direct control of LHRH secretion from the pituitary by LHRH agonists or antagonists.

Another group of chemical contraceptive agents are the local spermaticides, such as nonoxynol or octoxynol, which are placed into the vagina immediately prior to coitus in the form of creams, foams, jellies, or suppositories. The spermicidal action takes place either in the vagina or elsewhere in the reproductive tract. For the latter to occur, the spermicidal agent is either absorbed on sperm membranes or is transported into the uterus under the influence of uterine contractions. The spermicidal methods are not completely reliable in preventing pregnancy and are inconvenient to use.

From the foregoing, it is evident that the presently available methods of contraception are inadequate for various reasons. Although many women practice contraception in spite of these inadequacies, a need exists in medicine for new methods.

Pulmonary hypertension represents a serious, life threatening spectrum of diseases of multiple etiology. These include congenital abnormalities of the lung, thorax and diaphragm, congenital or acquired valvular or myocardial disease, obstructive lung disease, and can be a complication of autoimmune diseases, vasculitis and collagen based diseases (Rubin, Chest, 104: 236, 1993). Patients with pulmonary hypertension frequently present with symptoms including dyspnea, fatigue, syncope, and chest pain, and have increased pulmonary artery pressure and demonstrate prominence of the main pulmonary artery, hilar vessel enlargement and decreased peripheral vessels on chest radiographs (Rich, Ann. Internal. Med., 107: 216, 1987).

While pulmonary hypertension has multiple etiologies, primary pulmonary hypertension appears to involve an autoimmune component and has been reported as a complication in patients with mixed connective tissue disease, rheumatoid arthritis, Sjogren's syndrome, systemic sclerosis and lupus (Sato, Hum. Path., 24: 199, 1993). Primary pulmonary hypertension occurs in females 1.7 times more frequently than males with the greatest predominance between the third and fourth decades of life (Rich, Ann. Internal. Med., 107: 216, 1987). The increased incidence of primary pulmonary hypertension in women of child bearing age as well as the clinical observations that the disease can be exacerbated by pregnancy and oral contraceptives (Miller, Ann. Rheum. Dis., 46: 159, 1987; and cited in Farhat et al., J. PET., 261: 686, 1992) suggests a role for estrogen in the disease process. To this extent, Farhat et al. have demonstrated that estradiol potentiates the vasopressor response to a thromboxane mimetic in perfused rat lungs (J. PET., 261: 686, 1992). However, the role of estrogen in pulmonary hypertension is complex and may be dependent on the etiology of the disease process. In a rat model of pulmonary hypertension induced by injection of monocrotaline pyrrole (Reindel, Tax. Appl. Pharm., 106: 179, 1990) progressive pulmonary hypertension, right ventricular hypertrophy and interstitial edema around the large airways and blood vessels becomes apparent, similar to the pathology observed in man. Estradiol treatment decreased right ventricular hypertrophy and prevented interstitial edema in this model (Farhat et al., Br. J. Pharm., 110: 719, 1993) as well as attenuating the hypoxic vasoconstrictive response in isolated sheep lungs (Gordon et al., J. Appl. Physiol., 61: 2116, 1986).

Current therapy for pulmonary hypertension is inadequate and is largely dependent on the use of vasodilators, diuretics, and anticoagulants (Rubin, Drugs, 43: 37, 1992; Palevsky, JAMA, 265:1014, 1991). Vasodilators are effective in only a small subpopulation of patients with primary pulmonary hypertension and is complicated by systemic hypotensive responses. Prostacyclin infusion and high dose calcium channel blockers are also being used with limited efficacy. Heart-lung and single lung transplantation have been used on patients which do not respond to vasodilator therapy, however, due to surgical morbidity and mortality, this approach is usually limited to those patients who continue to deteriorate despite aggressive therapy at centers experienced in management of this disease. Patients frequently



die of right heart failure and those individuals which have signs of right heart failure have a mean survival of 6-12 months (Rubin, Drugs, 43: 37, 1992).

Therefore, pulmonary hypertensive diseases are characterized by inadequate therapies, necessity of organ transplantation and poor prognosis, and a need exists for new therapies.

Acne and seborrhea are two general classes of skin diseases which are marked by an abnormal function (usually hyperactivity) of the sebaceous glands in the skin. The subject of this invention is the use of compounds to inhibit acne and seborrhea.

Acne vulgaris is a disease of the pilosebaceous unit in the skin and is chronic and inflammatory in nature. It is characterized by comedos (blackheads), papules, pustules, cysts, and nodules. The areas of the body most commonly affected by the disease are those which have the most sebaceous glands, i.e., the face, neck, back, and chest. Acne is a very common disease in both men and women and usually appears at the beginning of puberty. Although the disease is usually mild and resolves itself by the time most people reach their midtwenties, it can in many instances be disfiguring and a source of great physiological distress. In some extreme cases, acne can be the source of severe infection and even life-threatening.

The etiology and pathogenesis of the disease begins with cohesive hyperkeratosis in which cornified cells adhere and block the follicular canal between the sebaceous gland and the surface of the skin. The sebaceous gland under hormonal control (testosterone and dihydrotestosterone) are stimulated to enlarge and produce increasing amounts of sebaceous secretions (principally in the form of triacylglycerols). These sebaceous secretions are trapped in the blocked, follicular canal and build up to form a closed comedo. At this stage, common, indigenous skin bacteria (principally, Propionibacterium Acnes) begin to metabolize the triacylglycerols to free fatty acids. These liberated fatty acids are inflammatory and results in the formation of a papule. This papule is often raised and is typical of an inflammatory lesion, i.e., red, edematous, and painful. The papule may continue to expand and rupture the follicle wall, thus forming a pustule or cyst. The pustule stage is very painful and unsightly and is often a site for secondary infection by opportunistic bacteria such as Staphylococcus. The pustules and cysts often lead to the scarring and disfigurement seen in severe cases of acne.

There are several drugs available for the treatment of acne. For mild cases, benzoyl peroxide is used and is often moderately effective. Benzoyl peroxide is thought to work by inhibiting cohesive hyperkeratosis and by suppressing P. Acnes. Although benzoyl peroxide is effective in mild cases of acne, it suffers from several drawbacks: first, it must be applied topically and does not always penetrate to the pilosebaceous unit where the acne lesion initiates, second, it can cause skin irritation which can exacerbate the disease. Another moderately effective drug is vitamin A (retenoic acid, Retin A) which is used topically. Vitamin A inhibits cohesive hyperkeratosis; however, being a topical preparation it suffers from some of the same drawbacks as benzoyl peroxide and in addition it can cause a deterioration of the protective stratum comeum if used extensively. Yet another group of commonly used drugs for the treatment of acne are antibiotics. These can be used either topically or systemically. The most commonly used antibiotics are tetracyclines and erythromycin and to a lesser extent minocycline, ampicillin, clindamycin, trimethoprim, and sulfamethoxazole. These antibiotics inhibit P. Acnes and other secondary bacterial infections. There are two major drawbacks to the prolonged use of antibiotics for acne; first, the continued long exposure to antibiotics often lead to formation of resistant bacterial strains both in the skin and systemically, and second continued use of antibiotics may lead to sensitization of the patient to the antibiotic. A newer drug used for acne is Isotretinoin (Accutane, 13-cis-retenoic acid). This drug works like vitamin A; however, it can be used systemically. The side-effects of isotretinoin are cheilitis, a rise in serum triglycerides, elevated sedimentation rates, and most importantly, isotretinoin is a teratogen in humans and therefore cannot be used if there is a question of pregnancy during treatment. All of the above drugs have some positive effect in the treatment of acne, but each has its limiting side-effects.

Hormonal therapy is also effective for the treatment of acne in women. In many cases, the administration of estrogens has a positive effect in treating acne. Estrogens counteract the effect of endogenous androgens and therefore, decrease sebaceous excretion. However, since the use of unopposed estrogen administration in women with a uterus poses the potential for the development of endometrial cancer, a cyclic therapy of estrogen and a progestin are used for the treatment of acne. Typically, women are prescribed the normal birth control protocols for acne treatment. Although, these protocols are often effective for acne, in many cases these regimens contain progestins which have significant androgenic activity. This androgenic activity exacerbates the disease. Additionally, it is well known that progestinal agents are the cause of many negative, psychological side-effects. Clearly, a better hormonal agent would be beneficial.

Seborrhea or seborrheic dermatitis is another group of skin diseases thought to be associated with abnormal function of the sebaceous glands. It occurs in areas where there are large numbers of sebaceous glands and is characterized by flashing of the skin and red, mildly inflammatory patches. Seborrhea is most common in the hair (a form of dandruff), scalp margins, eyebrows, naso-labial folds, external ear canals, postier auricular fold, and presternal area. Generally, mild seborrhea is controlled by topical medication such as glucocorticoids and LDH in Nivea oil. However, more severe cases are more difficult to control.

There are several conditions in which the ovaries do not develop and in consequence puberty does not occur. Gonadal dysgenesis results in a severe disease state known as Turner's Syndrome resulting from the absence of a second sex chromosome (X chromosome monosomy). The syndrome is associated with the female phenotype, shortness of stature, sexual infantilism, and various somatic abnormalities. Several typical features are observed in these patients including distinct facial features, square chest, and short broad neck with webbing. Additional anomalies include cubitus valgus, congenital lymphedema of the feet and hands, renal abnormalities, high arched palate, skeletal anomalies, pigmented nevi, keloid formation, abnormal nails, and recurrent otitis media. Cardiovascular abnormalities include bicuspid aortic valves, partial anomalous venous drainage, and hypoplastic left-sided heart syndrome (Miller, M.J., et al. *J. Pediatr.*, 102: 47-50, (1983), Mazzanti, I. et al. *Helv. Paediatr. Acta*, 43: 25-31, (1988), Van Egmond, H. et al. *Br. Heart J.*, 60: 69-71, (1988)). Renal abnormalities include rotation of the kidney, horseshoe kidney, duplication of renal pelvis and ureter, and hydronephrosis secondary to ureteropelvic obstruction.

Skeletal maturation is normal or slightly delayed in childhood but lags in adolescence as a result of gonadal steroid deficiency. Typically, fishnet appearance caused by localized rarefactions occurs. Bone mineral content reduction occurs as early as 8 years of age as well as later in puberty. Changes of the spine, vertebral hypoplasia, and scoliosis are also common. Abnormalities of the carpal, wrist, knee and pelvis are also noted. The shortness of stature, including uterine growth retardation, is not evident until after the first 3 years of life after which growth velocity decelerates appreciably (Park, E., et al. *Pediatr. Res.* 17: 1-7, (1983), Lyon, A. J. et al. *Arch. Dis. Child.*, 60: 932-935, (1985)). In general, the patients suffer from sexual infantilism with genital ducts and external genitalia being immature. As a result, ovarian development is retarded.

Current therapy is directed towards correcting stature, somatic anomalies and inducing secondary sexual characteristics. Recent data indicated growth hormone is a viable therapy for stature improvement (Rosenfeld, R. G., et al. *J. Pediatr.*, 113: 393 (1988)). Patients not treated with estrogen often develop a severe form of osteoporosis similar to that experienced by females after menopause. Fractures and vertebral collapse are common. Steroid hormone therapy is normally deferred until after 15 years of age as it is believed treatment at an earlier age may result in premature maturation of the skeleton and thus a decrease in height. In fact, pharmacological doses of estrogen can accelerate bone maturation and resulting in epiphyseal fusion at an early age without concomitant increases in height. Other studies have shown low-dose estrogen allows patients to develop breasts without causing any changes in height (Alexander, R.L. et al. *Clin. Res.* 26: 174A (1978)). However, studies indicate a number of cases of endometrial cancer in patients with gonadal dysgenesis as a result of estrogen therapy (Levine, L.S., *Pediatrics*, 62: 1178-1183 (1979)).

Given the adverse side effects of estrogen in Turner's Syndrome patients, a need exists for a bone sparing agent which does not possess significant uterotrophic consequences.

In the United States, one in every four women require medical attention for breast symptomatology. While much rarer, males also encounter breast disorders. Such disorders include galactorrhea, gynecomastia, hypertrophy, polythelia, mastodynia/mastalgia, hyperprolactinemia, and generally non-fibrocystic, non-cancerous mastopathias.

Breast pain is common and estimated to be present in 50% of women. Normally the etiology is unclear. The discomfort generally is classified as (1) cyclic mastalgia or mastodynia occurring immediately prior to the menses; (2) changes in the breast such as duct ectasia and sclerosing adenosis, or (3) referred pain such as costochondritis.

Gynecomastia is enlargement of the glandular breast tissue in male humans (the female counterpart is hypertrophy). This enlargement is localized to the areoles and can be unilateral or more commonly bilateral. The condition is usually benign in nature; however, it can be the source of severe psychological disturbance to the patient. Gynecomastia is most commonly found in males at the time of puberty, but can occur at any age. Gynecomastia can have many underlying causes, e.g., Klinefelter's syndrome (XXY chromosomal abnormality), liver disorders, estrogen therapy for prostatic carcinoma, tumors of various endocrine organs, and certain drugs (digitalis and Dilantin). The common relationship between all these causes and the resulting gynecomastia is the production of abnormal amounts of estrogens. Currently, treatment of this disease is limited to three therapies: 1) Determination and treatment of the underlying cause; 2) Surgical removal of the breast tissue; and 3) Treatment with diethylstilbestrol and radiation. Determination and treatment of the underlying cause of gynecomastia is not always possible. Surgery and treatment with diethylstilbestrol and radiation is not always successful and entails great expense and risk. Clearly, a more effective and safer therapy would be useful.

Galactorrhea is the production of breast milk in the male or female when not immediately associated with pregnancy. The highly inappropriate and rare response in the male breast is accompanied by severe psychological discomfort to the male patient. It is thought to be caused by an overproduction of estrogen and prolactin excess. Surgical treatment is usually the therapy of choice if the underlying cause can not be determined or treated. A safer and less costly therapy would be useful.

Diabetes mellitus is a systemic disease characterized by disorders in the actions of insulin and other regulatory hormones in the metabolism of carbohydrates, fats and proteins, and in the structure and function of blood vessels. The primary symptom of diabetes is hyperglycemia, often accompanied by glucosuria, the presence in urine of large amounts of glucose, and polyuria, the excretion of large volumes of urine. Additional symptoms arise in chronic or long

standing diabetes. These symptoms include degeneration of the walls of blood vessels. Although many different organs are affected by these vascular changes, the nerves, eyes and kidneys appear to be the most susceptible. As such, long-standing diabetes mellitus, even when treated with insulin, is a leading cause of blindness.

There are two recognized types of diabetes. Type I diabetes is of juvenile onset, ketosis-prone, develops early in life with much more severe symptoms and has a near-certain prospect of later vascular involvement. Control of this type of diabetes is difficult and requires exogenous insulin administration. Type II diabetes mellitus is of adult onset, ketosis-resistant, develops later in life, is milder and has a more gradual onset.

One of the most significant advancements in the history of medical science came in 1922 when Banting and Best demonstrated the therapeutic effects of insulin in diabetic dogs. However, even today, a clear picture of the basic biochemical defects of the disease is not known, and diabetes remains a serious health problem. It is believed that two percent of the United States' population is afflicted with some form of diabetes. The introduction of orally effective hypoglycemic agents was an important development in the treatment of hyperglycemia. Oral hypoglycemic agents are normally used in the treatment of adult onset diabetes.

Observations in animal models on glucose metabolism for type II diabetes and in humans suggest that sex steroids play a permissive role in the phenotypic expression of hyperglycemia. These observations have prompted studies on the effects of androgens and estrogens on blood glucose levels. Testosterone administration to intact or ovariectomized female rats resulted in marked insulin resistance which correlated to morphological changes in muscle, Holmang, et al., *Am. J. Physiol.*, **259**, E555-560 (1990); Holmang, et al., *Am. J. Physiol.*, **262**, E851-855 (1992). In streptozotocin diabetic rats, implanted testosterone antagonized the ability of residual insulin to maintain glycemic control, Le et al., *Endocrinology*, **116**, 2450-2455 (1985). In contrast, glucosuria disappeared in castrated diabetic KK mice and reappeared when androgens were replaced in these mice, Nonaka, et al., *Jpn. J. Vet. Sci.*, **50**, 1121-1123 (1988); Higuichi, et al., *Exp. Anim.*, **38**, 25-29 (1989).

Results from estrogen administrations also support the hypothesis that the balance between androgens and estrogens is critical to the development of hyperglycemia. Daily estradiol administrations to diabetic KK mice normalized the blood glucose levels and eliminated glucosuria, Toshiro, et al., *Jpn. J. Vet. Sci.*, **51**, 823-826 (1989). Estradiol also lowered the blood glucose levels of C57BL/6J-ob/ob mice, Dubuc, *Proc. Soc. Exp. Biol. Med.*, **180**, 468-473 (1985) and C57BL/KsJ-db/db mice, Garriss, *Anatomical Record*, **225**, 310-317 (1989).

In climacteric women, anxiety, depression, tension and irritability begin during the perimenopause and can be correlated to reduce estrogen levels and estrogen replacement therapy has been recommended for the treatment of these symptoms (Malleson J., *Lancet*, **2**: 158, (1953); Wilson et. al., *J. Am. Geriatric Soc.*, **11**: 347 (1963)). The mechanism for protective effects of estrogen in this case is unknown, but may be related to potential effects of estrogen on biogenic amines such as serotonin (Aylward M., *Int. Res. Communications System Med. Sci.*, **1**: 30 (1973)). To this regard circulating serotonin is reduced in post-menopausal women (Gonzales G., et. al., *Maturitas* **17**: 23-29 (1993)), and serotonin (as well as several other biogenic amines) have a putative role in behavioral depression.

Phillips and Sherwin (*Psychoneuroendocrinology*, **17**: 485-495 (1992)) reported that in surgically menopausal women given estrogen, scores in immediate and delayed recall tests are greater than in similar women not given estrogen. Two potential hypotheses might explain this effect. There is some evidence that partial estrogen agonists (or anti-estrogens) such as tamoxifen interact with the muscarinic receptor (Ben-Baruch G., et. al., *Molec. Pharmacol.*, **21**: 287-293 (1982)), and muscarinic agonists ( $M_2$ ) are known to produce positive effects in a number of memory associated tasks and may have clinical relevance in Alzheimer's Disease. Another interesting possibility may be linked to neuropeptides such as Substance P, which are known to have neurotrophic as well as memory-promoting effects (Thoenen H., *Trends in Neuroscience*, **14**: 165-170 (1991); Huston J. et. al., *Neurosci. Biobehav. Rev.*, **13**: 171-180 (1989)), thus, through an effect either at a neurotransmitter receptor in the CNS or at a neuropeptide receptor, a tissue selective estrogen agonist/antagonist could produce memory and cognitive enhancing effects. Such an activity would most relevantly be assessed in man, but a variety of animal models (i.e. maze learning, extinction etc.) are available for preclinical testing.

Perhaps the most frequent CNS related problem in climacteric women is the occurrence of hot flashes. While this undoubtedly is a somatic effect mediated by effects on the microvasculature, current evidence points strongly in the direction of CNS initiated effect (Lomax P., et. al., *Pharmac. Ther.*, **57**: 347-358 (1993)). Therefore, a tissue selective estrogen agonist/antagonist might offer the ideal therapy providing the desired effect in the absence of untoward side effects on reproductive tissue.

Obsessive-compulsive disorder is one of the rarer psychiatric illnesses, although minor obsessional symptoms probably occur in one-sixth of the population (*Encyclopedia of Medicine*, American Medical Association; *Current Diagnosis*, W.B. Saunders Company, 1985). It is characterized by one or both of two symptoms. The first comprises recurrent, intrusive ruminative thoughts that the patient may realize are senseless but of which he cannot stop thinking. The most common of these are thoughts of violence, contamination, doubt, or personal illness. Normally, the patient does not believe these thoughts are true reflections of reality. However, some patients become convinced that their obsessive ruminations are true, and suffer from psychotic delusions.

The second comprises repetitive, ritualistic behaviors that the patient recognizes are needless but that he cannot keep himself from performing. Hand washing, counting, checking rituals, and touching rituals are examples of such rituals. The carrying out of the ritual is not constant, but fluctuates and mirrors anxiety levels. There normally are intense feelings of panic and anxiety if the patient is prevented from completing a ritual.

While appearing depressed, a review of the history of obsessive-compulsive patients normally reveals that obsessions and compulsions precede the onset of dysphoric mood states and that depressed feelings are related to the impact the obsessive-compulsive behavior has on life. In severe cases, the patient will be incapacitated, completely overtaken by the distraction of constant obsessive ruminations or the demand to complete endless compulsive rituals.

Consumptive disorders include those disorders in which the intake, normally oral, of the amount of a substance is outside a normal range, often to an extent where health is impaired. Examples of such are eating or appetite disorders (obesity, bulimia, pica, anorexia nervosa, and psychogenic rumination) and substance abuse or overuse (smoking, nicotine dependence, alcoholism, alcohol abuse).

It is well known that the chronic administration of nicotine results in tolerance and, eventually, dependence. The use of tobacco has become extremely widespread in all countries, despite the well known adverse effects of the use of tobacco in all its forms. Thus, it is clear that tobacco use is extremely habit-forming, if not addictive, and that its use provides sensations to the user which are pleasant and welcome, even though the user is fully aware of the drastic long term ill effects of its use.

Cigarette smoking is the most dominant cause of preventable morbidity and early demise in developed countries. On average, smokers die several years earlier than nonsmokers and have an increased risk of fatal heart disease, lung cancer, cancers of the mouth, throat, esophagus, pancreas, kidney, bladder, and cervix, peptic ulcers and of fractures of the hip, wrist, and vertebrae. Olfaction and taste are impaired in smokers, and facial wrinkles are increased. Diabetic patients who smoke may have an increased risk of proteinuria.

Smoking cessation provides benefits, even late in life, such as reducing the risk of death or myocardial infarction in persons with coronary artery disease, reducing the progression of carotid atherosclerosis, and with reversal of chronic bronchitis.

Children of persons who smoke have lower birth weights, more frequent respiratory infections, less efficient pulmonary function, and a higher incidence of chronic ear infections than children of non-smokers and are more likely to become smokers themselves. Exposure to passive smoke has been shown to increase the risk of cervical cancer, lung cancer, and heart disease and to promote endothelial damage and platelet aggregation.

Recently, vigorous campaigns against the use of tobacco have taken place, and it is now common knowledge that the cessation of smoking brings with it numerous unpleasant withdrawal symptoms, which include irritability, anxiety, restlessness, lack of concentration, lightheadedness, insomnia, tremor, increased hunger and weight gain, and, of course, a craving for tobacco.

Alcohol abuse and alcohol dependence (i.e., alcoholism) are serious public health problems of modern society. In the United States alone, an estimated 13 million adults exhibit symptoms of alcohol dependence due to excessive alcohol intake, and an additional 7 million abuse alcohol without showing symptoms of dependence according to U.S. government projections from studies conducted in the mid-1980s. Alcohol dependence and abuse are very expensive as it is estimated that it cost the U.S. well over \$200 billion in 1991 with no prospect of falling or leveling off. The social and psychological damages inflicted on individuals as a consequence of alcohol abuse, e.g., children born with fetal alcohol syndrome (FAS) and victims of alcohol-related accidental death, homicide, suicide, etc., are immense.

While it is generally accepted that alcoholism and alcohol abuse are afflictions with staggering international economic, social, medical, and psychological repercussions, success in preventing or otherwise ameliorating the consequences of these problems has been an elusive goal. Only very recently the public view that alcoholism and alcohol abuse are remedial solely by moral imperatives has been changed to include an awareness of alcoholism and alcohol abuse as physiological aberrations whose etiology may be understood and for which therapy may be found through scientific pursuits. Both alcohol abuse and dependence arise as a result of different, complex, and as yet incompletely understood processes. At present, alcohol research is in the mainstream of scientific efforts.

This invention provides methods for inhibiting obsessive-compulsive and consumptive disorders.

Hirsutism (hypertrichosis) is characterized by excessive growth of hair. In women, hirsutism refers specifically to excessive growth of hair in a male pattern and distribution. Clinically, hirsutism in women is seen as a growth of terminal hair on the face (particularly on the upper lip), the chin, chest, back, and lower abdomen (escutcheon). This growth of hair is often seen as unsightly and can be the cause of embarrassment and psychological distress. Hirsutism is a common occurrence at the menopause, but can occur any time after puberty. The etiology of the condition has been linked to over production of androgens by either the ovaries or adrenal glands or both.

Hirsutism in women can be treated in a variety of ways. Cosmetic treatment of the condition, including shaving, plucking of hairs, and bleaching, while effective in improving the appearance of the patient, are only palliative and must be constantly reapplied. Glucocorticoid steroids are often effective; however, they have the potential of serious side-effects such as Cushing's Syndrome. Oral contraceptives can be effective; however, care must be taken because

certain progestins used in common oral contraceptive regiments may actually contribute hirsutism because of their androgenic side-effects. Clometidine and Spironolactone have shown some effectiveness in the treatment of hirsutism; however, each of these can have unwanted side-effects. Clearly, a more effective and better tolerated agent would be useful.

5 Alopecia (hair loss) can occur in women for a variety of reasons, and includes female pattern alopecia. Female pattern alopecia is characterized by chronic and progressive hair loss often beginning around thirty years of age and accelerating at menopause. The hair loss is usually confined to the central scalp in a diffuse pattern. This loss of hair is cosmetically damaging and often psychologically disturbing to the patient. The etiology of the condition has been linked to an elevated level of androgens and the subsequent response of androgen sensitive hair follicles. Treatment  
10 of the condition is primarily cosmetic in nature, e.g., wigs, hair styles which cover the effected area, etc. The drug, Spironolactone, has been used, but does have side-effects. Clearly, an effective treatment for this condition would be useful.

Macrophages play a central role in host defense through a variety of effector mechanisms involving both membrane related and secretory events (Gordon *et. al.*, *Curr. Opin. Immunol.*, 4, 25, 1992; Fuller, *Brit. Med. J.*, 48, 65, 1992).  
15 Phagocytosis, chemotaxis and antigen presentation are membrane related processes involved in immunologic defense mechanisms necessary for host survival. The importance of macrophages in defense against microbes, immune surveillance, destruction of tumor cells, and in the clearing of senescent erythrocytes has been documented in man and in animal models characterized by the selective elimination of macrophages (Claassen *et. al.*, *J. Immunol Meth.*, 134, 153, 1990). Macrophages also contribute to host defense through secretion of bacteriostatic and bactericidal proteins,  
20 cytokines and lipid mediators, as well as oxygen and nitrogen reactive intermediates. The secretory capacity of the macrophage is central to its function as these cells secrete over 100 distinct mediators and are located in every organ (Nathan, *J. Clin. Invest.*, 79, 319, 1987).

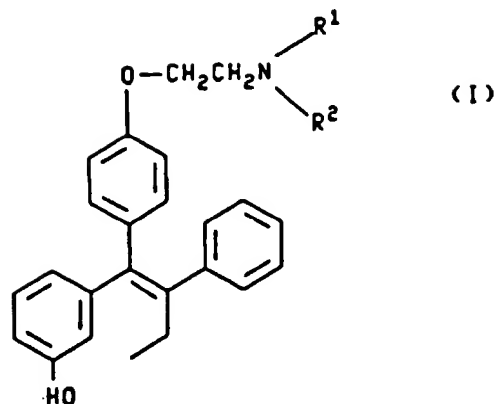
While aberrant activation of macrophage functions is associated with autoimmune diseases as well as both chronic and acute inflammatory processes, the reciprocal condition, suppression of macrophage effector functions, is associated  
25 with reoccurring infections of both opportunistic and non-opportunistic pathogens and contributes to increased morbidity and mortality. Populations associated with an immunocompromised state include burn patients, transplants, HIV infected individuals, cancer patients undergoing chemotherapy and surgical patients, notably those with a higher risk of infection as observed in thoracoabdominal patients.

Current therapeutic approaches to these patients includes the use of intravenous infusion of macrophage derived  
30 cytokines notably the colony stimulating factors G-CSF, GM-CSF, and M-CSF (Nemunaltis, Transfusion 33: 70, 1993). Supportive therapy with antibiotics and fluids is also used, however, the limitations of these approaches are demonstrated by the continued problems of infection in immunocompromised patients and the emergence of more deadly strains of antibiotics resistant organisms. Furthermore, infections of immunocompromised patients with opportunistic pathogens including Pneumocystis and Cryptococcal infections remain significant and result in complications despite  
35 various antibiotic protocols. Clearly, novel therapeutics which can selectively enhance macrophage effector functions to augment host defense would play a central role in the clinical management of these patients.

Estrogen has been reported to increase select macrophage effector functions including Fc mediated phagocytosis, class II antigen expression, and IL-1 secretion. These observations coupled with the known propensity of women to be more resistant to a variety of infections (Ahmed *et al.*, *Am. J. Path.*, 121, 531, 1985) suggests that estrogen-like  
40 compounds may enhance macrophage effector functions and thus be beneficial in disease states associated with depressed host defense such as bladder infections or depressed wound healing.

The term "inhibit" is defined to include its generally accepted meaning which includes prophylactically treating a subject to prevent the occurrence of one or more of these disease states, holding in check the symptoms of such a disease state, and/or treating such symptoms. Thus, the present methods include both medical therapeutic and/or  
45 prophylactic treatment, as appropriate.

The methods of this invention are practiced by administering to an individual in need of treatment an effective amount of a compound formula I



wherein

R<sup>1</sup> and R<sup>2</sup> may be the same or different provided that, when R<sup>1</sup> and R<sup>2</sup> are the same, each is a methyl or ethyl group, and, when R<sup>1</sup> and R<sup>2</sup> are different, one of them is a methyl or ethyl group and the other is hydrogen or a benzyl group; or a pharmaceutically acceptable salt thereof.

Compounds of formula I are known in the art and essentially are prepared via the methods described in U.S. Pat. No. 5,047,431, which is hereby incorporated herein by reference.

A preferred formula I compound is that in which R<sup>1</sup> and R<sup>2</sup> each are methyl. This preferred compound is known as droloxifene which previously has been described as an antiestrogenic agent and is useful for the treatment of hormone dependent mammary tumors (U.S. Pat. No. 5,047,431), and for the relief of bone diseases caused by the deficiency of estrogen or the like (U.S. Pat. No. 5,254,594). Furthermore, droloxifene is known to have less uterotrophic effect than other antiestrogenic compounds such as tamoxifen.

Although the free-base form of formula I compounds can be used in the methods of the present invention, it is preferred to prepare and use a pharmaceutically acceptable salt form. Thus, the compounds used in the methods of this invention form pharmaceutically acceptable acid and base addition salts with a wide variety of inorganic and, preferably, organic acids and include the physiologically acceptable salts which are often used in pharmaceutical chemistry. Such salts are also part of this invention. Typical inorganic acids used to form such salts include hydrochloric, hydrobromic, hydroiodic, nitric, sulfuric, phosphoric, hypophosphoric, and the like. Salts derived from organic acids, such as aliphatic mono and dicarboxylic acids, phenyl substituted alkanic acids, hydroxyalkanoic and hydroxyalkandioic acids, aromatic acids, aliphatic and aromatic sulfonic acids, may also be used. Such pharmaceutically acceptable salts thus include acetate, phenylacetate, trifluoroacetate, acrylate, ascorbate, benzoate, chlorobenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, methylbenzoate, o-acetoxybenzoate, naphthalene-2-benzoate, bromide, isobutyrate, phenylbutyrate, β-hydroxybutyrate, butyne-1,4-dioate, hexyne-1,4-dioate, caprate, caprylate, chloride, cinamate, citrate, formate, fumarate, glycollate, heptanoate, hippurate, lactate, malate, maleate, hydroxymaleate, malonate, mandelate, mesylate, nicotinate, isonicotinate, nitrate, oxalate, phthalate, terephthalate, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, propionate, propionate, phenylpropionate, salicylate, sebacate, succinate, suberate, sulfate, bisulfate, pyrosulfate, sulfite, bisulfite, sulfonate, benzenesulfonate, p-bromophenylsulfonate, chlorobenzenesulfonate, ethanesulfonate, 2-hydroxyethanesulfonate, methanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, p-toluenesulfonate, xylenesulfonate, tartarate, and the like. A preferred salt is the citrate salt.

The pharmaceutically acceptable acid addition salts are typically formed by reacting a compound of formula I with an equimolar or excess amount of acid. The reactants are generally combined in a mutual solvent such as diethyl ether or benzene. The salt normally precipitates out of solution within about one hour to 10 days and can be isolated by filtration or the solvent can be stripped off by conventional means.

The pharmaceutically acceptable salts of formula I compounds generally have enhanced solubility characteristics compared to the compound from which they are derived, and thus are often more amenable to formulation as liquids or emulsions.

Once prepared, the free base or salt form of formula I compounds can be administered to an individual in need of treatment for the methods herein described. The following nonlimiting test examples illustrate the methods of the present invention.

For the methods of the present invention, compounds of Formula I are administered continuously, or from 1 to 4 times daily.

As used herein, the term "effective amount" means an amount of compound of the methods of the present invention which is capable of inhibiting the symptoms of the pathological conditions herein described. The specific dose of a

compound administered according to this invention will, of course, be determined by the particular circumstances surrounding the case including, for example, the compound administered, the route of administration, the state of being of the patient, and the severity of the pathological condition being treated. A typical daily dose will contain a nontoxic dosage level of from about 0.25 mg to about 100 mg/day of a compound of the present invention. Preferred daily doses generally will be from about 1 mg to about 40 mg/day.

The compounds of this invention can be administered by a variety of routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, and intranasal. These compounds preferably are formulated prior to administration, the selection of which will be decided by the attending physician. Typically, a formula I compound, or a pharmaceutically acceptable salt thereof, is combined with a pharmaceutically acceptable carrier, diluent or excipient to form a pharmaceutical formulation.

The total active ingredients in such formulations comprises from 0.1 % to 99.9% by weight of the formulation. By "pharmaceutically acceptable" it is meant the carrier, diluent, excipients, and/or salt must be compatible with the other ingredients of the formulation, and not deleterious to the recipient thereof.

Pharmaceutical formulations containing a compound of formula I can be prepared by procedures known in the art using well known and readily available ingredients. For example, the compounds of formula I can be formulated with common excipients, diluents, or carriers, and formed into tablets, capsules, suspensions, powders, and the like. Examples of excipients, diluents, and carriers that are suitable for such formulations include the following fillers and extenders such as starch, sugars, mannitol, and silicic derivatives binding agents such as carboxymethyl cellulose and other cellulose derivatives, alginates, gelatin, and polyvinyl-pyrrolidone; moisturizing agents such as glycerol; disintegrating agents such as calcium carbonate and sodium bicarbonate agents for retarding dissolution such as paraffin resorption accelerators such as quaternary ammonium compounds; surface active agents such as cetyl alcohol, glycerol monostearate; adsorptive carriers such as kaolin and bentonite; and lubricants such as talc, calcium and magnesium stearate, and solid polyethyl glycols.

The compounds also can be formulated as elixirs or solutions for convenient oral administration or as solutions appropriate for parenteral administration, for example, by intramuscular, subcutaneous or intravenous routes.

Additionally, the compounds are well suited to formulation as sustained release dosage forms and the like. The formulations can be so constituted that they release the active ingredient only or preferably in a particular physiological location, possibly over a period of time. The coatings, envelopes, and protective matrices may be made, for example, from polymeric substances or waxes.

Compounds of formula I generally will be administered in a convenient formulation. The following formulation examples only are illustrative and are not intended to limit the scope of the present invention.

In the formulations which follow, "active ingredient" means a compound of formula I, or a salt thereof.

#### Formulation 1: Gelatin Capsules

Hard gelatin capsules are prepared using the following:

Ingredient	Quantity (mg/capsule)
Active ingredient	0.25-100
Starch, NF	0-650
Starch flowable powder	0-50
Silicone fluid 350 centistokes	0-15

A tablet formulation is prepared using the ingredients below:

#### Formulation 2: Tablets

Ingredient	Quantity (mg/tablet)
Active ingredient	0.25-100
Cellulose, microcrystalline	200-650
Silicon dioxide, fumed	10-650
Stearate acid	5-15

The components are blended and compressed to form tablets.



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Alternatively, tablets each containing 0.25-100 mg of active ingredient are made up as follows:

### Formulation 3: Tablets

Ingredient	Quantity (mg/tablet)
Active ingredient	0.25-100
Starch	45
Cellulose, microcrystalline	35
Polyvinylpyrrolidone (as 10% solution in water)	4
Sodium carboxymethyl cellulose	4.5
Magnesium stearate	0.5
Talc	1

The active ingredient, starch, and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders which are then passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50° - 60°C and passed through a No. 18 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 60 U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets.

Suspensions each containing 0.25-100 mg of medicament per 5 ml dose are made as follows:

### Formulation 4: Suspensions

Ingredient	Quantity (mg/5 ml)
Active ingredient	0.25-100 mg
Sodium carboxymethyl cellulose	50 mg
Syrup	1.25 mg
Benzoic acid solution	0.10 mL
Flavor	q.v.
Color	q.v.
Purified Water to	5 mL

The medicament is passed through a No. 45 mesh U.S. sieve and mixed with the sodium carboxymethyl cellulose and syrup to form smooth paste. The benzoic acid solution, flavor, and color are diluted with some of the water and added, with stirring. Sufficient water is then added to produce the required volume. An aerosol solution is prepared containing the following ingredients:

### Formulation 5: Aerosol

Ingredient	Quantity (% by weight)
Active ingredient	0.25
Ethanol	25.75
Propellant 22 (Chlorodifluoromethane)	70.00

The active ingredient is mixed with ethanol and the mixture added to a portion of the propellant 22, cooled to 30°C, and transferred to a filling device. The required amount is then fed to a stainless steel container and diluted with the remaining propellant. The valve units are then fitted to the container.  
Suppositories are prepared as follows:

## Formulation 6: Suppositories

Ingredient	Quantity (mg/suppository)
Active ingredient	250
Saturated fatty acid glycerides	2,000

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimal necessary heat. The mixture is then poured into a suppository mold of nominal 2 g capacity and allowed to cool.

An intravenous formulation is prepared as follows:

## Formulation 7: Intravenous Solution

Ingredient	Quantity
Active ingredient	20 mg
Isotonic saline	1,000 mL

The solution of the above ingredients is intravenously administered to a patient at a rate of about 1 mL per minute.

Antiestrogens are compounds that prevent estrogens from expressing their effects on estrogen dependent target tissues consequently antagonizing a variety of estrogen-dependent processes. However, most antiestrogens such as tamoxifen are not pure antagonists, since they exhibit some estrogenicity. The methods below enable the skilled practitioner to determine the estrogen and antiestrogen effect of the compounds of this invention. U.S. 4,859,585, incorporated herein by reference claims two alternative general protocols by which a substance may be characterized as an estrogen agonist and/or estrogen antagonist.

Methods to Determine Estrogenic and Antiestrogenic Potential

Uterine weight test. Compounds of Formula I are given orally to immature female Sprague-Dawley (SD) rats (20 days old; 40 g body wt; Charles River Wiga, Sulzfeld, F.R.G.) for 3 consecutive days to test estrogenic activity. In addition to each dose of a compound of formula I, a standard dose of 1 mg/kg estradiol is administered orally to juvenile SD rats to determine the antiestrogenic effect of the compounds. The compounds are suspended in 0.25% agar for the administration. The animals are killed on day 4, the uteri removed, cleared of any intrauterine fluid and subsequently weighed in a dry condition. Estrogenic activity is estimated by the increase in uterine weight. (uterotropic effect) initiated by the respective daily doses of compounds of formula 1. The antiestrogenic effect of the compounds is tested by the reduction of the uterine weight (anti uterotrophic effect) in the presence of 1 mg/kg estradiol.

Estrogen receptor-binding assay. Estrogen receptors (ER) are measured in the cytosol of uterine tissue of female immature white New Zealand rabbits (3 months of age). The uteri are separated from surrounding fatty tissue, rinsed in ice-cold phosphate-buffered saline, and immediately transferred into liquid nitrogen. The frozen uterine tissue is put into a capped Teflon cylinder pre-cooled in liquid nitrogen that is vibrated (501 {7.}) for at least 30 sec. in a microdis-membrator (Braun, Melsungen, F.R.G.) in the presence of a tungsten carbide ball. The resulting powder is mixed with units (1:4/w:v) of Trisbuffer (0.01 M Tris, 0.001 EDTA, pH 7.5), homogenized with a Dounce homogenizer and centrifuged at 105,000 g for 1 hr. The supernatant (cytosol) is decanted and the protein concentration adjusted to 5 mg protein/mL. The protein concentration is measured according to Lowry et al.[8]. Aliquots of cytosol are pipetted into plastic tubes,  $2.5 \times 10^{-9}$ M [ $^{125}$ I]-estradiol, and a range of concentrations of unlabeled estradiol and antiestrogens of formula I are added. The relative binding affinity of the antiestrogens to the estrogen receptor is carried out with the dextran-charcoal method at 2°C.

All steps are carried out in triplicate. The relative binding affinity is defined as the ratio of the concentrations of radioinert  $^{125}$ I-estradiol to the compound of formula I that is necessary to achieve a 50% inhibition of the specific [ $^{125}$ I]-estradiol binding. Bound radioactivity at the highest concentration of  $^{125}$ I-estradiol ( $2.5 \times 10^{-7}$ M) is taken as unspecific binding and subtracted from all values.

Procedures for evaluating compounds of this invention for treatment of skin and vaginal atrophy are described in U.S. Patent 5,461,064, which is incorporated herein by reference.

Skin Atrophy

Three to twenty women, who are post-menopausal and in good health, are selected. Additionally, these women are selected on the basis of their presenting several signs of rapid dermal atrophy, such as a rapid increase in the number of facial wrinkles or crow's feet, rapid change in the pigmentation of the skin, i.e. "age spots", or other complaints of rapid dermal aging. It should be remembered by the attending physician that these criterion may be highly subjective to the patient and that some consideration must be taken into account in patient selection. Also, dermal atrophy may be the result of other factors such as UV damage from the sun or other environmental insults and that such patients who are suffering from these effects would be excluded.

The first component of the study is qualitative and subjective one, i.e., an evaluation of improvement in the patient's appearance. Such an evaluation requires an initial benchmark for future comparison. Some initial benchmarks might be in the form of a standardized set of questions as to how the patient views her own appearance, photographs of the patient, or a psychological profile of the patient's self-image. The second component is quantitative; these include the measurement of urinary excretion of hydroxyproline, moisture content of the skin, glycosaminoglycans in the skin, and changes in resilience and pliability of the skin. Methods for determining these factors are found in "The Menopause", Ed. R.J. Beard, University Press, Chapter 7 (1977) and "Methods in Skin Research", Ed. Skerrow, D. and Skerrow C. J., John Wiley & Sons Ltd., Chp. 22, "Analysis of Sebaceous Lipids", p. 587-608 (1985), and further references cited therein, all herein incorporated by reference. Again, an initial benchmark of these quantitative factors is obtained.

The women, thus selected and initially evaluated, are placed in a clinical protocol of receiving 20-100 mg of a compound of this invention by oral administration either as a single or split dose. Alternatively, these patients are placed in a protocol for topical administration to areas of the skin most effected by the atrophy. This topical protocol includes the use of a suitable formulation containing 5-50% (by weight) of an active compound of this invention applied to the affected area once or twice a day. Either of these protocols continues two to twelve months. Subsequent evaluations, both quantitative and qualitative, are made at appropriate intervals.

A positive result is an improvement in the overall qualitative index of the patient's appearance and/or an improvement in the quantitative parameters, e.g., an increase in the urinary excretion of hydroxyproline signifying an increase in turnover and synthesis of collagen, an increase in moisture content, glycosaminoglycans, pliability, or resilience of the skin.

Vaginal Atrophy

Three to twenty women suffering from vaginal atrophy associated with menopause are selected. These women are in general good health. Since the nature of this disorder is highly idiosyncratic and subjective, evaluation of the effectiveness of treatment would necessarily be subjective in nature. These patients are asked to keep a daily log noting such details as vaginal itching and scaling and the degree of comfort in sexual intercourse. These women are placed on a clinical protocol similar to that described above for atrophy of the skin. Particular emphasis is placed on the use of vaginal suppositories containing 5-25% of an active compound of this invention.

A positive result is an improvement in the comfort of sexual intercourse and/or a decrease in vaginal itching or scaling.

Utility of the compounds described herein is exhibited by the positive results observed in one or both of the above assays.

Procedures for evaluating the utility of a compound of this invention for increasing the libido of post-menopausal women are described in United States Patent 5,439,931, which is incorporated herein by reference.

Assay 1

Animals used are ovariectomized or ovariectomized/adrenalectomized Sprague-Dawley rats (Specific Pathogen Free-Anticimex, Stockholm) weighing 250-300 gms. They are housed in a room maintained at a temperature of 24°C under reversed lighting (10 hours dark). Food and water (or saline) are available ad libitum. A compound of the invention is administered to one group of rats, and the other group is maintained as a control, and behavioral observations are made by placing each female with 2 cage-adapted, sexually experienced males for a 5-minute period during which about 20 mounts occur. The following measures are recorded:

1. Proportion of mounts by the male which elicited a lordosis response - L/M;
2. Lordosis intensity measured on a 3 point scale;
3. Lordosis duration (in seconds);
4. Female acceptance ratio - No. of mounts divided by No. of refused mounting attempts plus mounts, a measure of the female's willingness to accept the male when he attempts to mount her.

Activity of the compound is shown through positive impact on any one of the 4 observations, as compared to control.

## Assay 2

Five to fifty women are selected for the clinical study. These women are post-menopausal, i.e., have ceased menstruating for between 6 and 12 months prior to the study's initiation, are in good general health, and suffer from self-described loss of libido especially noted after menopause. Because of the idiosyncratic and subjective nature of this symptom, the study has a placebo control group, i.e., the women are divided into two groups, one of which receive the active agent of this invention and the other receives a placebo. Women in the test group receive between 50-200 mg of the drug per day by the oral route. They continue this therapy for 3-12 months. Accurate records are kept as to the level of libido of the women in both groups and at the end of study these results are compared.

Activity of the compounds of the invention are illustrated by positive effects in at least one of the above assays.

Test methods for measuring the ability of a compound of this invention to inhibit fertility in women are described in U.S. Patent 5,462,949, which is incorporated herein by reference.

## Assay 1

Between five and fifty young adult virgin female rats weighing 200-300 g. each are separated into groups having the same number of rats. One of the groups serves as the control group and the other groups as experimental groups, each such experimental group receiving raloxifene at a particular dose level. Raloxifene is prepared in corn oil such that the daily administration is in 0.1 ml. of vehicle. The designated quantity of raloxifene in the vehicle is administered to each rat within the defined group subcutaneously (sc) daily. Alternatively, administration may be made via oral gavage or an intramuscular route. The control group receives only the vehicle. Administration of the vehicle or the combination of raloxifene and vehicle is continued on a daily basis for 15 days. On the 5th day of treatment, one or two adult male rats weighing at least 250 g. are added to each group, and cohabitation is continued until the 15th day at which time the male rats are withdrawn from the group. Each group of female rats then is maintained for an additional seven days after which the rats are sacrificed and examined for the presence of viable or resorbing fetuses.

The number of animals that exhibit evidence of pregnancy over the number of animals in the group multiplied by one hundred is the pregnancy ratio percentage (PRP). A compound is considered active when the PRP is 0 to 20%. A PRP of 40% constitutes marginal activity, and anything higher is inactive.

## Assay 2

Between five and fifty young adult virgin female rats weighing 200-300 g. each are separated into groups having the same number of female rats, and paired with male rats. One of the groups serves as the control group and the other groups as experimental groups. Vaginal Smears are performed on the females daily until sperm and vaginal plugs are found, which coincides with the day of vaginal estrus and is designated day one of pregnancy.

The male rats are removed, and the experimental groups of female rats are administered raloxifene via oral gavage, an intramuscular route, or by subcutaneous injection. The administration continues on a daily basis until the twelfth day of pregnancy at which time all the female rats are sacrificed and examined for the presence of implantation sites. A compound is considered active when the PRP, as defined above, is 60% or lower.

Utility of the compounds described herein is exhibited by activity in at least one of the above assays.

Procedures for determining the effectiveness of compounds of this invention in treatment of pulmonary hypertensive disease are described in U.S. Patent 5,447,941, which is incorporated herein by reference.

## Assay 1

The procedure as set out in Farhat et al., J PET, 261: 686 (1992) (herein incorporated by reference) is carried out. Four to thirty rats are sacrificed. The lungs are exsanguinated by perfusion via the hepatic pulmonary vein. The pulmonary artery is cannulated as is the trachea to maintain ventilation and the pulmonary cannula is connected to the perfusion line and the whole ventilated lung is removed and suspended in a perfusion chamber. The effects of vasoconstrictor substances on perfusion pressure of the isolated perfused lung is measured using a Statham pressure transducer. The increase in perfusion pressure (vasoconstriction) induced by thromboxane mimetics in the presence of estradiol is determined and the ability to block the thromboxane effects with a compound of formula I or the estradiol potentiation of the thromboxane effects will be determined.

Activity of compounds of formula I is illustrated by a reduction in pulmonary perfusion pressure increase following thromboxane mimetic stimulation.

Assay 2

Between five and fifty rats are administered a single IV dose of monocrotaline pyrrole, (3.5 mg/kg) and pulmonary disease is evaluated by histopathology, accumulation of fluorescein conjugated dextran in bronchial alveolar lavage fluid (as a measurement of pulmonary edema), and measurement of artery pressure using a Standtham P231D pressure transducer (Reindel et al., Tax and Applic. Pharm. 106:179-200 (1990), incorporated herein by reference. A compound of formula I is administered and the effect on the rats are evaluated.

Activity of compounds of formula I is illustrated by a reduction in uptake of fluorescein conjugated dextran from bronchial alveolar lavage fluids of animals treated with a compound of formula I, indicating a reduction in pulmonary edema. Rat lungs will also be removed from thorax, perfused with modified Karnovskys fixative and processed for histopathology. A reduction in thickening of the arterial walls in treated rats is evidence for the protective role of compounds of formula I as is a decrease in pulmonary artery pressure.

Assay 3

Five to fifty women are selected for the clinical study. The women suffer from a pulmonary hypertensive disease. Because of the idiosyncratic and subjective nature of these disorders, the study has a placebo control group, i.e., the women are divided into two groups, one of which receives a compound of formula I as the active agent and the other receives a placebo. Women in the test group receive between 50-200 mg of the drug per day by the oral route. They continue this therapy for 3-12 months. Accurate records are kept as to the number and severity of the symptoms in both groups and at the end of the study these results are compared. The results are compared both between members of each group and also the results for each patient are compared to the symptoms reported by each patient before the study began.

Utility of the compounds of formula I is illustrated by the positive impact they have in at least one of the assays described above.

The utility of a compound of this invention for inhibiting acne or seborrhea is tested by the procedures described in U.S. Patent 5,439,923, incorporated herein by reference.

Assay 1

Each of from between two and twenty patients selected for the clinical evaluation is placed in a comfortable environment, i.e., comfortable temperature, humidity, lighting, etc. These patients have refrained from strenuous exercise and consumption of spicy foods for the twelve hours prior to the evaluation. An area of the body which contains a large number of sebaceous glands affected by seborrhea or acne, such as the forehead, is wiped with a gauze pad to remove accumulated lipids. A patch of the skin is taped off, forming a rectangle sized 2.5 by 1.8 cm. A pad of cigarette paper or other suitable absorbent material sized 2.5 by 1.8 cm is placed on the test area of the skin. The absorbent material must have first been defatted with ether prior to the placement on the test area to remove background lipids. The pad is then held in place with a bandage. After fifteen minutes the pad is replaced with a fresh pad (test pad). This procedure removes the background lipids in the skin so the true rate of lipid production by the sebaceous glands may be determined. The test pad is left in place for three to six hours and then removed. The test pad is then extracted with ether to remove the lipids and the ether evaporated. The residual lipids are then weighed. The result is expressed as the number of sebaceous lipids (mg) per 10 cm<sup>2</sup> per hour. The patient then takes either 30-400 mg/day of the active ingredient by the oral route, or applies a topical formulation containing 5-20% by weight of the active ingredient daily, both for three to nine weeks. The above described test pad methodology is repeated several times throughout administration of the active ingredient to monitor progress. This assay may also be performed on animals to verify utility. A positive effect is reflected by a decrease of the rate of sebaceous gland lipid production.

Assay 2

Between two and twenty patients are enrolled in this clinical protocol and are initially evaluated by direct observation of the skin and lesions thereon. This is done by choosing one cm<sup>2</sup> sections of affected skin and the number and type of lesion (comedos, seborrheic lesions, etc.) is noted. The areas normally used are the cheeks, scalp or back. The patient then takes either 30-400 mg/day of the active ingredient by the oral route, or applies a topical formulation containing 5-20% by weight of the active ingredient daily, both for three to nine weeks. The areas of the skin being evaluated are checked during the period of administration. Care must be taken to evaluate the same areas and in order to accomplish this, a small mark or marks may be made on the skin by a permanent marker. A positive result is reflected by a reduction in the number and/or severity of the lesions in the monitored areas of the skin.

Utility of the compounds described herein is exhibited by the positive results observed in one or both of the above

assays.

Utility of the compounds of this invention for treating Turner's Syndrome is determined by a procedure described in U.S. Patent 5,441,966, incorporated herein by reference.

## 5 Test Procedure

Five to thirty females are selected for the clinical study. The females are between the age of twelve and eighteen and exhibit characteristics of Turner's Syndrome, but are in good general health otherwise. The study has a placebo control group, i.e., the females are divided into two groups, one of which receives the active agent of this invention and the other receives a placebo. Females in the test group receive between 10-100 mg of the active agent per day by the oral route. They continue this therapy for 3-12 months. Accurate records are kept as to the number and severity of the above mentioned symptoms in both groups and at the end of the study these results are compared. The results are compared both between members of each group and also the results for each patient are compared to the symptoms reported by each patient before the study began.

Utility of the compounds of the invention is illustrated by the positive impact they have on one or more of the symptoms when used in a study as above.

EP 0 659 419 A1 incorporated herein by reference provides methods for evaluating compounds of the present invention for breast disorders.

## 20 Assay 1

Five to fifty women are selected for the clinical study. The women have a history of a breast disorder as described herein, but are in good general health. Because of the subjective nature of these disorders, the study has a placebo control group, i.e., the women are divided into two groups, one of which receive the active agent of this invention and the other receive a placebo. Women in the test group receive between 50-200 mg of the drug per day by the oral route. They continue this therapy for 3-12 months. Accurate records are kept as to the status of the breast disorders in both groups and at the end of the study these results are compared. The results are compared both between members of each group and also the results for each patient are compared to the symptoms reported by each patient before the study began.

Utility of the compounds of the invention is illustrated by the positive impact they have on the disorder or a symptom or symptoms thereof when used in a study as above.

## Assay 2

Between three and twenty male patients suffering from gynecomastia or galactorrhea are selected. Initial measurement of breast size and evidence of lactation is noted. The patients receive 30-100 mg of an active compound of this invention per day as a single or divided dose via the oral route. This treatment is continued for 3-12 months. At appropriate intervals, further measurements of breast size or evidence of lactation are being made.

Utility of the compounds of the invention is illustrated by the positive impact on the disorder or its symptoms.

A method for determining hypoglycemic activity of the compounds of this invention is set forth in EP 0 635 264 A2, incorporated herein by reference.

Five to 6 month old male, inbred viable yellow obese-diabetic mice are used. Male viable yellow mice are obese, hyperglycemic, hyperinsulinemic and insulin resistant.

Mice are housed 6 per plastic cage with bedding and fed water and Purina Formulab Chow 5008 (Purina Mills, St. Louis, MO) ad libitum. The temperature of the animal rooms is maintained at  $23 \pm 2^\circ\text{C}$ . Lights in the animal rooms were on from 0600 to 1800 h.

Antiestrogens are tested at various doses as admixtures of diets. Each dose of an antiestrogen is tested on 6 mice housed in the same cage. Compounds are mixed in pulverized chow and repelletized. Mice serving as controls are given repelletized diet without any test compound. Blood samples are collected from the tail vein immediately before and weekly after the start of a test. Blood glucose concentrations are determined by the glucose oxidase method with a model 300 Alpkem Rapid Flow Analyzer (Clackamas, OR).

Reduction of blood glucose concentration below the levels of the control is indicative of an effective antiestrogen with utility in treatment of diabetes and hyperglycemia.

The effect of compounds of the present invention on central nervous system (CNS) disorders in post-menopausal women may be evaluated by a method described in EP 0 659 413 A2, incorporated herein by reference.

Test Procedure

Five to fifty women are selected for the clinical study. The women are post-menopausal, i.e., have ceased menstruating for between 6 and 12 months prior to the study's initiation, are in good general health, and suffer from one or more of the above-mentioned CNS disorders. Because of the idiosyncratic and subjective nature of these disorders, the study has a placebo control group, i.e., the women are divided into two groups, one of which receive the active agent of this invention and the other receive a placebo. Women in the test group receive between 50-100 mg of the drug per day by the oral route. They continue this therapy for 3-12 months. Accurate records are kept as to the number and severity of the above mentioned disorders in both groups and at the end of the study these results are compared. The results are compared both between members of each group and also the results for each patient are compared to the disorders reported by each patient before the study began.

Utility of the compounds of the invention is illustrated by the positive impact they have on one or more of the CNS symptoms/disorders when used in a study as above.

Methods for evaluating the effect of compounds of this invention in treating obsessive-compulsive and consumptive disorders are described in EP 0 659 428 A1, incorporated herein by reference.

Assay 1

In order to demonstrate the *in vivo* effect of the compounds on alcohol consumption, experiments are designed to test the effect on free choice ethanol intake in golden hamsters. Hamsters are chosen based on previous reports that they are receptive to and give preference to high ethanol intake when compared with several other mammalian species. Kulkosky and Comell (*Pharmacol. Biochem. & Behav.* 11: 439-44, 1979) concluded that the species differences in ethanol intake and preferences were correlated with differences in ethanol metabolism.

The animals used for the experiments described herein are two to six male adult golden hamsters. Animals are maintained on a light/dark cycle of 14 hours of light per day and for a 6-week acclimation period. Animals have access to food and water *ad libitum*.

For the experiment, the animals are maintained as described above in a single large cage with four 250 ml calibrated drinking bottles. The drinking bottles are fitted with stainless steel straight sipper tubes used to measure fluid consumption to the nearest 5 ml. Spillage from the drinking tubes is caught by 2 oz. jars fitted with glass funnels and positioned under the sipper tubes. Fluid consumption by the hamsters is measured once every 3 days so that the consumption volumes are large enough to obtain reasonably accurate measurements.

After a 6-week acclimation period, the body weights of the animals are taken, and water intake is noted. Water in 2 of the 4 drinking bottles is then replaced by a 15% ethanol solution and consumption of water and aqueous ethanol are measured for a period of 2 weeks. Within 2 to 3 days after the beginning of this free choice phase of feeding, the hamsters establish an explicit preference for aqueous ethanol over water and the initial preference ratio (aqueous ethanol intake divided by water intake) is noted.

As a control, the animals are then fed with 0.2 ml water twice daily, using a stainless steel animal feeding needle. Water feeding does not seem to have any effect on the animals' drinking behavior as measured by total fluid intake. After 6 days, the same group of hamsters are fed a compound of formula I via a liquid mixture for a period of 3 to 12 weeks. Activity of the compounds of formula I is illustrated by the preference ratio being lower during administration of said compound of the invention than the initial preference ratio.

Assay 2

Five to fifty women are selected for the clinical study. The women are postmenopausal, i.e., have ceased menstruating for between 6 and 12 months prior to the study's initiation, are in good general health, and suffer from either obsessive-compulsive or a consumptive disorder. Because of the idiosyncratic and subjective nature of these disorders, the study has a placebo control group, i.e., the women are divided into two groups, one of which receives raloxifene as the active agent and the other receives a placebo. Women in the test group receive between 50-200 mg of the drug per day by the oral route. They continue this therapy for 3-12 months.

Accurate records are kept as to the number and severity of the symptoms in both groups and at the end of the study these results are compared. The results are compared both between members of each group and also the results for each patient are compared to the symptoms reported by each patient before the study began.

Utility of the compounds of formula I is illustrated by the positive impact they have on one or more of the disorders/symptoms when used in an assay described above.

EP 0 659 414 A2, incorporated herein by reference describes methods for evaluating compounds of the invention for inhibition of hirsutism and alopecia.



Hirsutism

Three to twenty women suffering from hirsutism are selected. These patients are initially scored for the extent and severity of hirsutism. The clinical evaluation is made by the methods described in the reference "Methods of Skin Research," John Wiley and Sons, pp 308-318 (1985), and the references cited therein. The patients receive 10-400 mg of an active compound of this invention per day as a single or split dose by oral administration. Alternatively, they apply a 10%, by weight of active ingredient, creme or lotion once or twice a day to the affected areas. The patient continues this protocol for six months. At appropriate intervals, re-evaluation by one of the methods described above would be made.

Alopecia

Three to twenty women suffering from female pattern alopecia are selected. These patients are initially scored for the extent and severity of the alopecia. This clinical evaluation is made by the methods described in "Methods of Skin Research," John Wiley and Sons, pp 308-318 (1985) and Habif, T., "Clinical Dermatology," C. V. Mosby Co., Chapter 23, pp 493-504 (1985); and references therein, herein incorporated by reference. Especially helpful in these evaluations is the "hair pluck" procedure and measurement of anagen to telogen ratio. The patients receive 10-400 mg of an active compound of this invention per day as a single or split dose by oral administration. Alternatively, the patients apply a 5-10% (by weight of a compound of this invention) as a creme, lotion, or shampoo to the affected area, once to twice a day. This protocol continues for six months. At appropriate intervals, re-evaluation by one of the methods described in the above references is made. A positive result is exhibited by an increase in the anagen to telogen ratio or an increase in the number of terminal hairs in the affected scalp region.

Utility of the compounds of the invention is illustrated by the positive impact they have on one or more of the symptoms when used in a study as above.

Assays which show the ability of compounds of the invention to increase macrophage function are described in EP 0 659 425 A1, incorporated herein by reference.

Assay 1

The procedure as set out in Friedman *et al.*, *J. Clin. Invest.*, **75**, 162-167 (1985) (herein incorporated by reference) is carried out, with certain modifications. Between five and one hundred mice are administered oral doses in the range of 1-10 mg/kg of a compound of formula 1 on a daily basis. Following the administration, macrophages are harvested and changes in both immune (Fc mediated) and non-immune phagocytosis are quantitated by using fluorescein conjugated yeast particles prepared based on Ragsdale, *J. Immunol. Meth.*, **123**:259 (1989). For immune mediated phagocytosis, fluorescein conjugated yeast is preincubated with mouse sera to promote opsonization. Increase in fluorescence uptake by macrophages is quantitated by an increase in fluorescent emission using excitation and emission wavelengths of 482 and 520 nm, respectively. This procedure is used with ex vivo or in vitro macrophage cultures and changes in fluorescence units quantitated.

An increase in fluorescent units, as compared to control indicates activity of compounds of formula 1.

Assay 2

The procedure as set out in Zuckerman *et al.*, *Cell immunol.*, **103**:207, (1986); *J. Immunol.*, **140**:978 (1988) (herein incorporated by reference) is carried out. The ability to induce class II antigens and consequently promote antigen presentation is determined on ex vivo primary peritoneal macrophages and in vitro with the murine macrophage cell line P388D1. Between five and one hundred mice are dosed with a compound of formula 1 macrophages are harvested and probed with antibodies against class II antigens of the D haplotype. Increased class II antigen expression is determined by flow cytometry using the appropriate secondary antibodies. In vitro studies evaluate the effects of the compounds in increasing the basal level and gamma interferon inducible expression of class II antigen by flow cytometry. An increase in class II expression reflect an increase in macrophage activation.

Assay 3

The procedure as set out in Seow *et al.*, *J. Immunol. Meth.*, **98**, 113 (1987) (herein incorporated by reference) is carried out. The assay is used to evaluate increases in macrophage effector functions which uses measurements of 2-deoxyglucose uptake. Macrophages ex vivo and in vivo are plated in 96 well plates at  $10^5$  cells per well and incubated in phosphate buffered saline in the presence of 0.78 uCi/ml of 3H-deoxyglucose, and a compound of formula 1 is placed in the wells. Reduction in the amount of extracellular glucose reflects the uptake of this non-metabolizable

glucose analog and consequently provides an independent assay for the determination of the state of macrophage activation mediated by the compound of formula 1. Increase in deoxyglucose uptake by the compound demonstrates the ability of the compounds to increase the state of macrophage activation.

#### 5 Assay 4

The procedure as set out in Zuckerman, Circ Shock, 29, 279 (1989) (herein incorporated by reference) is carried out to illustrate the ability of the compounds of formula 1 to protect in murine sepsis and endotoxin lethality models. Between five and one hundred mice are dosed orally with 1-10 mg/kg with a compound of formula 1 for 1 week prior to sepsis challenge. Challenge is performed using a bolus IV endotoxin injection under condition in which an LD100 is achieved (200 µg lipopolysaccharide). Exogenous glucocorticoids such as dexamethasone at 20 mg/kg serve as a positive control in creasing survival. The effects of the compound of formula 1 is also determined using a sepsis model involving cecal ligation and puncture. Sepsis by both Gram positive and Gram negative organisms results in an LD100 by 48 hours despite the use of antibiotics. An increase in the number of surviving animals or in survival time, as compared to control, demonstrates the activity of the compounds.

#### Assay 5

The ability of the compounds of formula 1 to increase the secretion of cytokines such as TNF is quantitated in vivo by sera measurements using commercially available TNF EUSAs specific for mouse TNF. Between five and one hundred mice are orally dosed with 1-10 mg/kg of a compound of formula 1 for one week prior to injection of a lethal or sublethal dose of lipopolysaccharide (200 and 1 µg, respectively). At one hour post LPS injection the mice are bled and the basal and LPS inducible amounts of serum TNF determined. Routinely, TNF levels below 10 pg/ml are observed prior to LPS injection and achieve levels of 5-20 ng/ml following LPS. The ability of the compounds to modulate the basal or inducible levels of TNF is determined. An increase in basal TNF without triggering massive systemic TNF release in compound treated mice demonstrates the activity of the compounds in promoting cytokine secretion. Finally, ex vivo and in vitro measurements of TNF release from peritoneal macrophages exposed to 1-5 µM of a compound in vitro is also performed by EUSA to determine the extent of cytokine increase mediated by a compound of formula 1.

#### 30 Assay 6

Five to fifty women are selected for the clinical study. The women are immunosuppressed. Because of the idiosyncratic and subjective nature of these disorders, the study has a placebo control group, i.e., the women are divided into two groups, one of which receives a compound of formula 1 as the active agent and the other receives a placebo. Women in the test group receive between 50-200 mg of the drug per day. They continue this therapy for 3-12 months. Accurate records are kept as to the number and severity of the symptoms in both groups and at the end of the study these results are compared. The results are compared both between members of each group and also the results for each patient are compared to the symptoms reported by each patient before the study began.

Utility of the compounds of formula 1 in increasing macrophage function is illustrated by the positive impact they have in at least one of the assays described above. Such compounds are useful in combating infections and promote wound healing.

#### TEST PROCEDURE FOR PMS

Three to fifty women are selected for the clinical study. The women have regular menses, are in good general health, and suffer from one or more of the above mentioned PMS/LLPDD symptoms. Because of the somewhat idiosyncratic and subjective nature of these symptoms, the study has a placebo control group, i.e., the woman are divided into two groups, one of which receives the active agent of this invention and the other receives a placebo. Women in the test group receive between 10-100 mg of the drug per day by the oral route. They continue this therapy for 1-3 months. Accurate records are kept as to the number and severity of the symptoms in both groups and at the end of the study these results are compared. The results are compared both between members of each group and also the results for each patient are compared to the symptoms reported by each patient before the study began. See U.S. Patent 5,389,670.

Utility of the compounds of the invention for inhibiting the symptoms of PMS/LLPDD is illustrated by the positive impact they have on one or more of the symptoms when used in a study as above.

TESTS FOR UTERINE FIBROSISTest 1

Between 3 and 20 women having uterine fibrosis are administered a compound of the invention. The amount of compound administered is 0.1 to 100 mg/day, and the period of administration is 3 months.

The women are observed during the period of administration and up to 3 months after discontinuance of the compound for effects on uterine fibrosis.

Test 2

The same procedure is used as in Test 1, except the period of administration is 6 months.

Test 3

The same procedure is used as in Test 1, except the period of administration is 1 year.

Test 4A. Induction of fibroid tumors in guinea pig.

Prolonged estrogen stimulation is used to induce leiomyomata in sexually mature female guinea pigs. Animals are dosed with estradiol 3-5 times per week by injection for 2-4 months or until tumors arise. Treatments consisting of a compound of the invention or vehicle is administered daily for 3-16 weeks and then animals are sacrificed and the uteri harvested and analyzed for tumor regression.

B. Implantation of human uterine fibroid tissue in nude mice.

Tissue from human leiomyomas are implanted into the peritoneal cavity and or uterine myometrium of sexually mature, castrated female nude mice. Exogenous estrogen are supplied to induce growth of the explanted tissue. In some cases the harvested tumor cells are cultured in vitro prior to implantation. Treatment consisting of a compound of the invention or vehicle is supplied by gastric lavage on a daily basis for 3-16 weeks and implants are removed and measured for growth or regression. At the time of sacrifice, the uteri is harvested to assess the status of the organ.

Test 5

A. Tissue from human uterine fibroid tumors is harvested, and maintained in vitro as primary nontransformed cultures. Surgical specimens are pushed through a sterile mesh or sieve or alternately teased apart from surrounding tissue to produce a single cell suspension. Cells are maintained in media containing 10% serum and antibiotics. Rates of growth in the presence and absence of estrogen are determined. Cells are assayed for their ability to produce complement component C3 and their response to growth factors and growth hormone. In vitro cultures are assessed for their proliferative response following treatment with progestins, GnRH, a compound of the invention and vehicle. Levels of steroid hormone receptors are assessed weekly to determine whether important cell characteristics are maintained in vitro. Tissue from 5-25 patients is utilized.

Activity in at least one of the above tests indicates the compounds of the invention are of potential in the treatment of uterine fibrosis.

ASSAYS FOR INHIBITION OF MYELOPEROXIDASEAssay 1

In order to investigate the myeloperoxidase activity inhibiting properties of compounds of formula 1, Assays 1 and 2, as set out in Jansson (*supra.*), are employed.

In this assay, human PMN leukocytes are stimulated with estriol to increase myeloperoxidase activity in the presence of added hydrogen peroxide. The conversion of luminol by hypochlorous acid is measured by chemiluminescence. The reaction mix consists of cells ( $10^6$ ), agent or compound of formula 1 ( $1\mu\text{M}$ ), hydrogen peroxide (0.1 mM), and luminol (0.2 mM) incubated at  $37^\circ\text{C}$ .

Estrogen and its analogs stimulate myeloperoxidase activity. Compounds of formula 1 antagonize the estriol stimulated chemiluminescence.

Assay 2

Purified human myeloperoxidase is incubated with agent (estrogen or a compound of formula 1), in the presence of luminol at 37°C. The substrate, hydrogen peroxide, is added and the chemiluminescence measured. The reaction mix is human MPE (250 ng), agent or a compound of formula 1 (10 µM, titrated), hydrogen peroxide (1 mM), and luminol (0.2 mM).

Estrogen and its analogs have little or no effect on MPE activity, however, compounds of formula 1 reduce the activity of purified MPE.

Assay 3

Five to fifty women are selected for the clinical study. The women suffer from SLE or rheumatoid arthritis. Because of the idiosyncratic and subjective nature of these disorders, the study has a placebo control group, i.e., the women are divided into two groups, one of which receives a compound of formula 1 as the active agent and the other receives a placebo. Women in the test group receive between 50-100 mg of the drug per day by the oral route. They continue this therapy for 3-12 months. Accurate records are kept as to the number and severity of the symptoms in both groups and at the end of the study these results are compared. The results are compared both between members of each group and also the results for each patient are compared to the symptoms reported by each patient before the study began.

Utility of the compounds of formula 1 is illustrated by the positive impact they have in at least one of the assays described above.

ASSAYS FOR INHIBITION OF ANTI-IMMUNE DISEASESAssay 1

The procedure as set out in Holmdahl et al., Clin. Exp. Immunol., 70, 373-378 (1987) (herein incorporated by reference) is carried out. Four to thirty female mice, aged approximately 8-10 weeks, are ovariectomized. Administration of a compound of the invention is begun within three weeks after castration on the experimental group. After one week of administration of a compound of formula 1, the mice are immunized with rat type II collagen. The mice are graded for clinical severity of arthritis, as set out in Holmdahl et al., Arthritis Rheum., 29, 106 (1986), herein incorporated by reference. Sera are collected, and assayed for anti-type II collagen reactive antibodies. At the termination of the experiment, spleen cells are obtained from the mice for determination of T cell activity.

Activity is illustrated by a reduction in titer of anti-collagen type II antibodies determined by conventional EUSA assay. Reduction in T-cell reactivity to type II collagen presented to splenic T-cells by antigen presenting cells is evaluated by quantitation of DNA synthesis by thymidine uptake. Finally, clinical severity of disease is evaluated daily by defining first signs of erythema and swelling of one or more limbs. Clinical assessment is correlated with histologic examination.

Assay 2

Between four and thirty young adult female Sprague-Dawley rats are fed animal chow and water ad libitum. The experimental group receives a compound of formula 1, and all rats receive rat cord generally as described in Amason et al., Arch. Neurol., 21, 103-108 (1969), incorporated herein by reference. The rats are graded for signs of experimental allergic encephalomyelitis (EAE). Between three and seven weeks after administration of a compound formula 1 began, the rats are sacrificed, their spinal cords removed and examined.

Activity is illustrated by the ability of a compound to inhibit EAE.

Assay 3

Between five and fifty mice (MRL/lpr and NZB) are used. Reduction of anti-DNA antibodies, quantitated by EUSA, as well as changes in survival time and histologic exam of kidneys are evaluated parameters. The mice are dosed with compounds of the invention and are evaluated using the above parameters for disease progression.

Assay 4

Five to fifty women are selected for the clinical study. The women are post-menopausal, i.e., have ceased menstruating for between 6 and 12 months prior to the study's initiation, suffer from an autoimmune disease which exhibits

symptoms, but otherwise are in good general health. Because of the idiosyncratic and subjective nature of these disorders, the study has a placebo control group, i.e., the women are divided into two groups, one of which receives a compound of formula 1 as the active agent and the other receives a placebo. Women in the test group receive between 50-200 mg of the drug per day by the oral route. They continue this therapy for 3-12 months. Accurate records are kept as to the number and severity of the symptoms in both groups and at the end of the study these results are compared. The results are compared both between members of each group and also the results for each patient are compared to the symptoms reported by each patient before the study began.

Utility of the compounds of formula I is illustrated by the positive impact they have in at least one of the assays described above.

#### ASSAY FOR PROTECTION AGAINST REPERFUSION DAMAGE

##### Assay

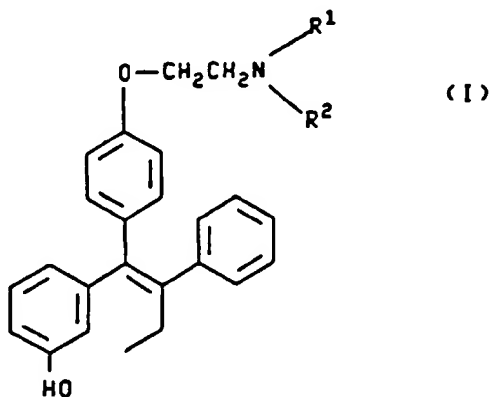
Ten male and ten female rabbits are treated with 1 mg of a compound of formula I. After 15 minutes, the rabbits are anesthetized and the coronary arteries are occluded for 30 minutes followed by four hours of reperfusion.

Comparable control groups are treated with vehicle.

The risk zone is assessed by blue dye and the infarct zone by tetrazolium staining. A reduced size of infarct zone relative to the control shows that the compound of formula I is effective in inhibiting reperfusion damage to ischemic myocardium.

#### **Claims**

1. The use of a compound of the formula (I) or of a pharmaceutically acceptable salt thereof for the manufacture of a medicament for inhibiting a pathological condition which is susceptible or partially susceptible to inhibition by an estrogen, antiestrogen or estrogen agonist, said pathological condition being uterine cancer, adjuvant breast cancer, breast disorders, male breast cancer, migraine, incontinence, vaginal atrophy, bladder infection, senile gynecomastia, diabetes, hyperglycemia, failure of wound healing, melanoma, impotence, inflammatory bowel disease, CNS and GI disorders caused by an excess of tachykinins, decreased libido, immune system disorders, decreased fertility, pulmonary hypertensive disease, acne, seborrhea, autoimmune disease, Turner's syndrome, alopecia, hirsutism, disorders related to an excess of neurokinin or an obsessive-compulsive disorder including smoking and alcohol abuse:-

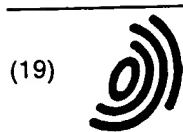


wherein

R<sup>1</sup> and R<sup>2</sup> may be the same or different provided that, when R<sup>1</sup> and R<sup>2</sup> are the same, each is a methyl or ethyl group, and, when R<sup>1</sup> and R<sup>2</sup> are different, one of them is a methyl or ethyl group and the other is hydrogen or a benzyl group

2. A use according to claim 1 wherein the compound of formula I is a compound wherein R<sup>1</sup> and R<sup>2</sup> each are methyl; or a pharmaceutically acceptable salt thereof.
3. A use according to claim 2 wherein said salt thereof is the citrate salt.

4. A use of claim 1 wherein said pathological condition is a breast disorder.
5. A use of claim 1 wherein said pathological condition is vaginal atrophy.
- 5 6. A use of claim 1 wherein said pathological condition is a bladder infection.
7. A use of claim 1 wherein said pathological condition is senile gynecomastia.
8. A use of claim 1 wherein said pathological condition is diabetes.
- 10 9. A use of claim 1 wherein said pathological condition is hyperglycemia.
10. A use of claim 1 wherein said pathological condition is failure of wound healing.
- 15 11. A use of claim 1 wherein said pathological condition is decreased libido.
12. A use of claim 1 wherein said pathological condition is an immune system disorder.
13. A use of claim 1 wherein said pathological condition is decreased fertility.
- 20 14. A use of claim 1 wherein said pathological condition is pulmonary hypertensive disease.
15. A use of claim 1 wherein said pathological condition is acne.
- 25 16. A use of claim 1 wherein said pathological condition is seborrhea.
17. A use of claim 1 wherein said pathological condition is autoimmune disease.
18. A use of claim 1 wherein said pathological condition is Turner's Syndrome.
- 30 19. A use of claim 1 wherein said pathological condition is hirsutism.
20. A use of claim 1 wherein said pathological condition is alopecia.
- 35 21. A use of claim 1 wherein said pathological condition is an obsessive-compulsive disorder.
22. A use of claim 1 wherein said pathological condition is undesired pregnancy.
- 40 23. The use of a compound of the formula (I) or of a pharmaceutically acceptable salt thereof as defined in any one of claims 1 to 3 for the manufacture of a medicament for inhibiting the symptoms of premenstrual syndrome.
24. The use of a compound of the formula (I) or pharmaceutically acceptable salt thereof as defined in any one of claims 1 to 3 for the manufacture of a medicament for inhibiting uterine fibrosis.
- 45 25. The use of a compound of the formula (I) or pharmaceutically acceptable salt thereof as defined in any one of claims 1 to 3 for the manufacture of a medicament for inhibiting myeloperoxidase activity.
26. The use of a compound of the formula (I) or pharmaceutically acceptable salt thereof as defined in any one of claims 1 to 3 for the manufacture of a medicament for inhibiting autoimmune diseases.
- 50 27. The use of a compound of the formula (I) or pharmaceutically acceptable salt thereof as defined in any one of claims 1 to 3 for the manufacture of a medicament for inhibiting reperfusion damage in ischemic myocardium.



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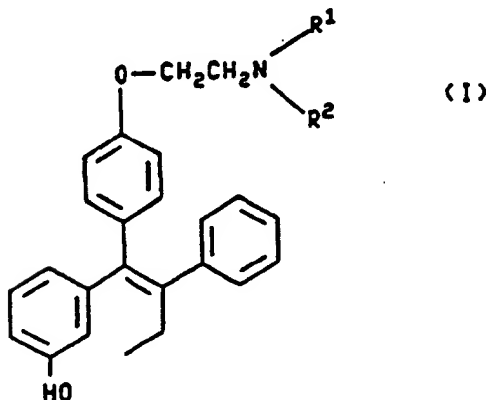
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(54) **Use of (E)-1-(4'-(2-alkylaminoethoxy)-phenyl)-1-(3'-hydroxyphenyl)-2-phenylbut-1-enes for inhibiting pathological conditions**

(57) The use of a compound of the formula (I) for the manufacture of a medicament for inhibiting a condition selected from pathological conditions related to organ systems which respond to estrogen agonists, uterine fibrosis, myeloperoxidase activity, autoimmune diseases, reperfusion damage in ischemic myocardium, and the symptoms of premenstrual syndrome:-

wherein

R<sup>1</sup> and R<sup>2</sup> may be the same or different provided that, when R<sup>1</sup> and R<sup>2</sup> are the same, each is a methyl or ethyl group, and, when R<sup>1</sup> and R<sup>2</sup> are different, one of them is a methyl or ethyl group and the other is hydrogen or a benzyl group.



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## EUROPEAN SEARCH REPORT

Application Number  
EP 97 30 1149

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
D,Y	EP 0 054 168 A (KLINGE PHARMA GMBH & CO.) 23 June 1982 * the whole document *	1-3	A61K31/135
Y	MURPHY, L.C. ET AL: "Mechanisms of growth inhibition by antiestrogens and progestins in human breast and endometrial cancer cells" THE JOURNAL OF STEROID BIOCHEMISTRY, vol. 43, no. 1, 1992, pages 117-121, XP000672947 * page 118, right-hand column, line 9-36 *	1-3	
Y	HORN, D.W. ET AL: "Effect of the nonsteroidal antiestrogen ZK 119.010 on growth and metastasis of the EndA endometrial carcinoma" INTERNATIONAL JOURNAL OF CANCER, vol. 58, no. 3, 1994, pages 426-429, XP000672680 * the whole document *	1-3	
Y	FUJIMOTO, J. ET AL: "Estrogen activates migration potential of endometrial cancer cells through basement membrane" TUMOR BIOLOGY, vol. 17, no. 1, January 1996, pages 48-57, XP000672945 * the whole document *	1-3	A61K
A	HOLINKA CF ET AL: "Effects of hormones on endometrial cancer cells in culture." ANN N Y ACAD SCI, 1991, 622 P422-38, UNITED STATES, XP000672114 * the whole document * * especially page 427, last line-page 428, line 3 *	1-3	
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)
-The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 30 May 1997	Examiner MAIR J.
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document			

EPO FORM 1503 03.82 (P04C01)



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Application Number

EP 97 30 1149

#### CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

- ☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claim(s):
- ☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

#### LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

- ☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.
- ☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:
- ☒ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:

1-3 partially



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# EUROPEAN SEARCH REPORT

Application Number  
EP 97 30 1149

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
D,A	EP 0 681 835 A (ELI LILLY AND COMPANY) 15 November 1995 * the whole document *	1-3	
D,A	WO 96 02243 A (PFIZER INC.) 1 February 1996 * the whole document *	1-3	
			TECHNICAL FIELDS SEARCHED (Int.Cl.6)
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 30 May 1997	Examiner MAIR J.
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons &amp; : member of the same patent family, corresponding document</p>			

EPO FORM 1503 03.82 (P04C01)



European Patent Office

EP97301149 - B -

**LACK OF UNITY OF INVENTION**

Annex to EP97301149:

The problem underlying the application as it is defined in claim 1 and the description is to provide alternative treatment for "a pathological condition which is susceptible to inhibition by an estrogen, antiestrogen or estrogen agonist", specifically, uterine cancer, "adjuvant breast cancer", breast disorders, male breast cancer, migraine, incontinence, vaginal atrophy, bladder infection, senile gynecomastia, diabetes, hyperglycemia, failure of wound healing, melanoma, impotence, inflammatory bowel diseases, CNS and GI disorders caused by an excess of tachykinins, decreased libido, immune system disorders, decreased fertility, pulmonary hypertensive disease, acne, seborrhea, autoimmune disease, Turner's syndrome, alopecia, hirsutism, disorders related to an excess of neurokinin or an obsessive-compulsive disorder including smoking and alcohol abuse. The proposed solution is the provision of medicaments containing a compound of formula (I).

Thus, in the context of the alleged invention, the use of pharmaceutical compositions containing a compound of formula (I) for the treatment of "a pathological condition which is susceptible to inhibition by an estrogen, antiestrogen or estrogen agonist" is the alleged contribution over the prior art and the special technical feature which may, a priori, unify the plurality of different inventions.

The use of a compound of formula (I) for the treatment of "a pathological condition which is susceptible to inhibition by an estrogen, antiestrogen or estrogen agonist" has been previously disclosed. See for example EP A 0 054 168, page 2, line 2-4.

Since a solution based on a technical feature identical to that proposed in the present application has already been disclosed this technical feature can no longer be accepted as the special technical feature involved in the technical relationship between the different inventions (according to Rule 30(1) EPC). In the absence of any other special technical features which could fulfil this role the requirements of Rules 30(1) and 30(2) EPC have not been met and there is no single inventive concept underlying the plurality of different inventions of the application.

Consequently there is a lack of unity a posteriori and the different inventions are formulated as the different subjects on the communication pursuant to Rule 46 EPC.

To search all these inventions would have required major additional searching effort.



European Patent Office

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**LACK OF UNITY OF INVENTION**

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions, or groups of inventions, namely:

Subject searched:

1. YES The use of a compound of formula (I) for the manufacture of a medicament for the treatment of uterine cancer. Claims 1-3 (partially).
2. NO The use of a compound of formula (I) for the manufacture of a medicament for the treatment of breast disorders including "adjuvant breast cancer", male breast cancer and senile gynecomastia. Claims 1-3 (partially), 4 and 7.
3. NO The use of a compound of formula (I) for the manufacture of a medicament for the treatment of migraine. Claims 1-3 (partially).
4. NO The use of a compound of formula (I) for the manufacture of a medicament for the treatment of incontinence, vaginal atrophy and a bladder infection. Claims 1-3 (partially), 5 and 6.
5. NO The use of a compound of formula (I) for the manufacture of a medicament for the treatment of diabetes and hyperglycemia. Claims 1-3 (partially), 8 and 9.
6. NO The use of a compound of formula (I) for the manufacture of a medicament for the treatment of failure of wound healing. Claims 1-3 (partially) and 10.
7. NO The use of a compound of formula (I) for the manufacture of a medicament for the treatment of melanoma. Claims 1-3 (partially).
8. NO The use of a compound of formula (I) for the manufacture of a medicament for the treatment of impotence and decreased libido. Claims 1-3 (partially) and 11.
9. NO The use of a compound of formula (I) for the manufacture of a medicament for the treatment of inflammatory bowel disease. Claims 1-3 (partially).
10. NO The use of a compound of formula (I) for the manufacture of a medicament for the treatment of CNS and GI disorders caused by an excess of tachykinins. Claims 1-3 (partially).
11. NO The use of a compound of formula (I) for the manufacture of a medicament for the treatment of immune system disorders and autoimmune disease. Claims 1-3 (partially), 12, 17 and 26.
12. NO The use of a compound of formula (I) for the manufacture of a medicament for the treatment of decreased fertility. Claims 1-3 (partially) and 13.
13. NO The use of a compound of formula (I) for the manufacture of a medicament for the treatment of pulmonary hypertensive disease. Claims 1-3 (partially) and 14.
14. NO The use of a compound of formula (I) for the manufacture of a medicament for the treatment of acne and seborrhea. Claims 1-3 (partially), 15 and 16.
15. NO The use of a compound of formula (I) for the manufacture of a medicament for

**LACK OF UNITY OF INVENTION**

- the treatment of Turner's syndrome. Claims 1-3 (partially) and 18.
16. NO The use of a compound of formula (I) for the manufacture of a medicament for the treatment of alopecia and hirsutism. Claims 1-3 (partially), 19 and 20.
17. NO The use of a compound of formula (I) for the manufacture of a medicament for the treatment of disorders related to an excess of neurokinin. Claims 1-3 (partially).
18. NO The use of a compound of formula (I) for the manufacture of a medicament for the treatment of an obsessive-compulsive disorder. Claims 1-3 (partially) and 21.
19. NO The use of a compound of formula (I) for the manufacture of a medicament for the treatment of undesired pregnancy. Claims 1-3 (partially) and 22.
20. NO The use of a compound of formula (I) for the manufacture of a medicament for the treatment of premenstrual syndrome. Claims 1-3 (partially) and 23.
21. NO The use of a compound of formula (I) for the manufacture of a medicament for the treatment of uterine fibrosis. Claims 1-3 (partially) and 24.
22. NO The use of a compound of formula (I) for the manufacture of a medicament for inhibiting myeloperoxidase activity. Claims 1-3 (partially) and 25.
23. NO The use of a compound of formula (I) for the manufacture of a medicament for the treatment of reperfusion damage in ischemic myocardium. Claims 1-3 (partially) and 27.
24. NO The use of a compound of formula (I) for the manufacture of a medicament for the treatment of smoking and alcohol abuse.